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Quantifying Impacts of Climate Change on Species Interactions While Fostering Undergraduate Research Experiences Using the Monarch (Danaus Plexippus)- Milkweed (Asclepias Sp.) System

Matthew J. Faldyn
Louisiana State University and Agricultural and Mechanical College, mfaldy1@gmail.com

QUANTIFYING IMPACTS OF CLIMATE CHANGE ON SPECIES INTERACTIONS WHILE FOSTERING UNDERGRADUATE RESEARCH EXPERIENCES USING THE MONARCH (*DANAUS PLEXIPPUS*)- MILKWEED (*ASCLEPIAS* SP.) SYSTEM

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ABSTRACT

Species interactions, specifically plant-insect interactions, are ubiquitous worldwide. Climate change will alter species interactions by affecting abiotic conditions, affecting species phenologies, interaction strengths, and physiological development. However, climate change impacts are often studied using individual species, with limited consideration quantifying the direct and indirect impacts of climate change species interactions. Using lab, field, and greenhouse experiments, I investigated how climate change will directly and indirectly affect species interactions while also fostering undergraduate research experiences using the monarch butterfly (*Danaus plexippus*)-milkweed (*Asclepias* sp.) system.

In North America, a widely planted, invasive milkweed species, *Asclepias curassavica*, negatively impacts monarch butterflies. I conducted a fully-factorial field experiment quantifying the indirect impacts of climate change on monarchs, as mediated through the invasive *A. curassavica* and native *A. incarnata*. Here, an ecological trap may be developing, driven by lethal increases in milkweed toxicity. Monarchs reared on the invasive *A. curassavica* at ambient conditions experienced improved performance, but under increased temperatures, monarchs fared much worse. Additionally, I conducted lab and field experiments to quantify the direct impacts of climate change on monarch butterflies and their protozoan parasite, *Ophryocystis elektroscirrha* (OE). OE threatens monarch populations by decreasing monarch performance, and empirical support is lacking on assessing the impacts of climate change on the interaction between parasites

and hosts. Here, simultaneous parasite infection and increased temperatures act as a one-two punch for monarchs, decreasing development time, weights, melanism, and size.

I also designed a course-based undergraduate research experience (CURE) for early-division undergraduate students. Here, enrolled students conducted a fully-factorial, greenhouse competition experiment between invasive *A. curassavica* and two native milkweed species, *A. incarnata* and *A. tuberosa*. CURE student performance to that of upper-division students enrolled in a traditional ecology laboratory was also assessed. We found that *A. curassavica* is a commensal competitor, and that CURE participation can effectively educate and engage early division students in conducting scientific research. In summary, my dissertation highlights the importance of empirically testing the direct and indirect impacts of climate change on species and their interactions, while reinforcing that novel course structures can foster scientific inroads for early division undergraduate students.

CHAPTER 1
INTRODUCTION

CLIMATE CHANGE IMPACTS

Climate change will affect the functioning of our planet by altering soil carbon sequestration and agricultural practices (Lal 2004), stressing global food security (Godfray *et al.* 2010), acidifying the oceans (Doney *et al.* 2009), raising sea-levels (Solomon *et al.* 2009), increasing the intensity and altering the frequency of extreme weather events (Easterling *et al.* 2000), increasing rates of species extinctions (Thomas *et al.* 2004), and inducing range shifts across species while altering species phenologies (Walther *et al.* 2002, Parmesan and Yohe 2003, Guisan and Thuiller 2005). These impacts are driven by changes in abiotic conditions, such as temperature, cycling of atmospheric CO_2, and pH (Walther *et al.* 2002, Karl *et al.* 2009). Ultimately, species must respond to these changes in through ecological and evolutionary adaptations (Parmesan 2006). For example, many species are undergoing rapid advances in phenologies along with latitudinal and altitudinal shifts, from plant species in Europe and North America flowering and unfolding their leaves earlier and European amphibians breeding earlier to New Zealand tree-lines advancing towards higher altitudes and Costa Rican lowland birds increasing altitudinal ranges (Walther *et al.* 2002). Additionally, while species may respond to environmental changes by altering their ecological and evolutionary responses, climate change induced environmental variation that is too rapid may not allow for species responses to compensate, leading to species incurring negative fitness costs or facing ecological traps (Schlaepfer *et al.* 2002, Fletcher *et al.* 2012). For example, increasing heat

waves in tropical regions increase tide pools temperatures past the upper tolerance limit of coastal species. Despite these species having a relatively high thermal limit, they have a low acclimation response, indicating a climate-induced trap may be developing (Vinagre *et al.* 2018). Furthermore, increased temperatures and anthropogenic structures (e.g., barns, houses) may act as an ecological trap for avian species, wherein these structures act as attractive nesting sites early in the breeding season but result in low breeding success due to high temperatures (Imlay *et al.* 2019). Thus, climate change has already impacted species and their interactions with abiotic and biotic environmental factors, and will continue to do so into the future.

Yet, empirical studies on the impacts of climate change on species are done with individual species based on classical analytical approaches designed for highly controlled experiments (Hobbs and Hilborn 2006). Additionally, quantifying the impacts across entire ecological communities can be difficult due to a variety of confounding factors (Legendre *et al.* 2002) and consideration for assessing climate impacts on species interactions using appropriate experimental and statistical designs is crucial (Brown *et al.* 2011). To address these issues and others, employing a model system using specialist species, or species with tightly-linked interactions between a host and consumer (e.g., plant-insect), can act as small-scale community modules that elucidate confounding interactions found at the community level (Holt and Polis 1997, Ali and Agrawal 2012). To this end, species interactions (e.g., using specialist, community modules) must be considered to fully understand how climate change will impact ecological dynamics (Parmesan and Yohe 2003, O'Connor *et al.* 2012, Urban *et al.* 2013).

SPECIES INTERACTIONS

Species interactions form the backbone of ecological communities (Dunson and Travis 1991). Interacting species are when a pair of organisms (living within an ecological community) affect one another, and can include interactions ranging from plant-insect interactions, host-parasite interactions, and species competition (among many others) (Cornell and Lawton 1992). One such species interaction that is ubiquitous worldwide are plant-insect interactions (Tylianakis *et al.* 2008). Insect herbivores are expected to be directly and indirectly effected by climate change because of their tight relationships with host plants, with cascading impacts in insect population and community dynamics along with changes in ecosystem functioning (Cornelissen 2011). Changes in temperature, CO_2, rainfall, and weather alter plant biochemistry and defense responses, having cascading impacts on insect fecundity, feeding, survival, population size, and dispersal (Jamieson *et al.* 2012, Trebicki *et al.* 2017). Broadly, these changes in host plant quality negatively affect insects, but some species possess compensatory responses, highlighting the complexity of these interactions and need for continued study (Trebicki *et al.* 2017). To this end, investigations into how climate change may impact insect species, specifically Lepidoptera, are of crucial importance (Woiwod 1997). Insects, especially lepidopteran species, act as remarkable model systems to study plastic responses because they are diverse, abundant, and have well-studied ecological interactions (Valtonen *et al.* 2011).

Climate change will not only impact traditional plant-herbivore interactions, but also host-parasite interactions, which serve as an important structuring interaction for ecological communities (Dobson and Hudson 1986, Altizer *et al.* 2013). Climate change

induced changes in abiotic conditions and biotic interactions is predicted to alter host behavior, contact rates, encounters with infective stages, births, deaths, and host immune defenses (Altizer *et al.* 2006, Rohr *et al.* 2011). For example, the pollen specialist bee (*Osmia iridis*) experiences improved activity under increased temperatures, but any activity benefit is canceled out by an increase in the activity of a brood wasp parasite (Forrest and Chisholm 2017). Additionally, leopard frogs (*Lithobates pipiens*) can develop physiological malformations from a parasitic trematode (*R. ondatrae*) infection, where warmer temperatures alter the timing of interactions between the two species leading to non-linear responses (Altizer *et al.* 2013). Yet, climate change induced impacts on parasites and their interactions with host species remain by-and-large empirically untested (Cizauskas *et al.* 2017). Ultimately, climate change is going to affect the antagonistic, commensalistic, and mutualistic interactions between species, alter food webs, increase the infectivity of pathogens, dampen plant mutualisms, and increase rates of herbivory while variably affecting rates of predation (Tylianakis *et al.* 2008).

MONARCH-MILKWEED STUDY SYSTEM

To address a need for empirical studies quantifying the impacts of climate change on species interactions, I employ the monarch butterfly (*Danaus plexippus*) – milkweed (*Asclepias* sp.) system to assess how climate change affects plant-insect and host-parasite interactions, while also addressing species competition.

Monarch butterflies are an extremely charismatic species, well-known to the public through their use for teaching biological processes in K-12 education (Matthews *et al.* 1997, Eick 2012), their accessibility to citizen scientists (Howard *et al.* 2010), listing as

an important pollinator species (Brower *et al.* 2006), and for their captivating, multi-generational, 3,500km annual migration (Brower and Malcolm 1991). Monarch butterflies have a wide distributional range across North America, spanning from central Canada through central Mexico, with isolated populations in the Caribbean and Hawaii (Altizer and Davis 2010). While Eastern migratory monarchs make the annual continental migration across North America, sedentary populations of monarch butterflies have established on the milkweed, *Asclepias curassavica*, in Florida, Texas, and Louisiana (Satterfield *et al.* 2015). A variety of environmental factors have been noted to affect monarch performance, such as water availability (Andrews and Hunter 2015), nutrient deposition (Zehnder and Hunter 2008), atmospheric CO_2 (Vannette and Hunter 2014), with climate change forcing monarch niches northward (Lemoine 2015) and negatively altering overwintering site precipitation (Oberhauser and Peterson 2003). With this in mind, consideration for how biotic interactions, for example with the monarch host plant, *Asclepias* sp., and their specialist parasite, *Ophryocystis elektroscirrha*, is crucial to disentangle how climate change may impact ecological communities. To this end, the monarch butterfly system acts as a strong candidate as a community module as they are specialist species, meaning the feed on either a single species (or multiple species within a specific genus), with their fitness dependent on the quality of their host resource (Ali and Agrawal 2012) and the top-down, biotic pressures with their parasite.

Asclepias sp., or milkweed plants, are the preferred host plant of monarch butterflies, with varying production of cardenolides, or secondary chemical defenses, of which monarchs sequester and store as an anti-predator defense (Brower *et al.* 1967) and

anti-parasite defense (de Roode et al. 2008). Like monarch butterflies, milkweed plants have a wide distributional range across the United States (Woodson 1954, Urquhart and Urquhart 1978). *Asclepias* sp. differ from one another in leaf morphology (Agrawal *et al.* 2009a), general phenologies (Woodson 1954), and in their two main types of herbivore defenses; latex exudation (Agrawal and Konno 2009) and cardenolide production (de Roode *et al.* 2008). Cardenolides are toxic, steroidal compounds that disrupt the Na+/K+ ATPase system in cell membranes, having the greatest impact on cardiac cell functionality (Malcolm 1991, Agrawal *et al.* 2012). Monarch fitness varies non-linearly with cardenolide production, where 'goldilocks' (i.e., intermediate) levels result in the highest conferred benefits to monarch fitness, with high levels of cardenolide production becoming too toxic for monarchs, but too little cardenolide production confers limited defensive benefit to monarchs (Malcolm 1994, Sternberg *et al.* 2012). Thus, any changes in milkweed quality will have cascading effects on monarch butterflies.

My dissertation employs three distinct species of milkweed; the invasive *A. curassavica*, and two native species, *A. incarnata* and *A. tuberosa*. The exotic, perennial milkweed, *A. curassavica,* is preferentially planted and sold across the Southeastern United States and is native to South America (Woodson 1954). Overall, this species can be 36-times more toxic than native milkweed species (Malcolm and Zalucki 1996) and negatively impacts monarch butterflies by reducing migratory propensity, increases parasite infection rates, and acts as an ecological trap (Satterfield *et al.* 2015, Faldyn *et al.* 2018). In contrast, *A. incarnata* is a Louisiana native, herbaceous, perennial milkweed species found throughout the Southeastern United States monarch migratory range that

produces low levels of toxic cardenolides and senesces it's leaves in the winter (Woodson 1954, Ladner and Altizer 2005). *A. tuberosa*, another native species, is found extensively throughout the Midwestern United States and forms a deep, woody rhizobial rootstalk with crowded, irregular, trichome-covered leaves (Woodson 1954). Due to rapid environmental change, overwintering and migratory range habitat loss, and increasing parasite (*Ophryocystis elektroscirrha*) infection across populations, monarch butterflies have experienced historic population declines (Belsky and Joshi 2018).

Monarch butterflies are not only affected by bottom-up effects from changes in their host plants, but also from top-down pressures such as parasite infection. Monarch butterflies are infected with the specialist parasite species, *Ophryocystis elektroscirrha*, or OE (McLaughlin and Myers 1970). OE infection reduces monarch survival, eclosion success, wingspan length, reproductive success, and overall activity (McLaughlin and Myers 1970, Altizer and Oberhauser 1999). OE is easily passed to monarch butterflies through vertical and horizontal transmission (Altizer *et al.* 2004). As monarchs develop, they typically consume OE-spores either on the egg chorion or on milkweed leaf tissue (McLaughlin and Myers 1970), where the parasite develops and replicates in their larval gut and emergent butterflies then begin shedding parasite spores (Altizer and Oberhauser 1999). The life-cycle of OE in tandem with monarch develop places additional stress on monarch butterflies, complicating and negatively impacting monarch growth (McLaughlin and Myers 1970, Altizer and Oberhauser 1999).

Ultimately, the tightly-linked interactions between the monarch butterfly, it's milkweed host plant, and it's neogregarine parasite, act as a refined community-module

with which to study the impacts of large-scale environmental perturbations (i.e., climate change) on ecological communities.

DISSERTATION SYNOPSIS

In Chapter 2, I assessed the impact of climate on plant-insect interactions. Specifically, I quantifying the indirect effects of climate on monarch butterflies as mediated through their milkweed host plant using open-top chambers (OTCs) to increase temperatures in experimental plots. Plots were placed with either invasive *Asclepias curassavica* or native *A. incarnata*, and monarch larvae. I hypothesized that if *A. curassavica* quality (e.g., a decrease in toxicity) were to improve due to environmental change, populations of sedentary, non-migratory monarchs could experience improved performance. Yet, if the quality of *A. curassavica* foliage were to decline under environmental change (e.g., toxic cardenolide production increases), sedentary monarch populations could fall into an ecological trap.

In Chapter 3, I quantified how climate change will affect host-parasite interactions, specifically using the monarch butterfly- OE system. I investigated how does elevated temperature and parasite infection affect monarch population dynamics and overall fitness. In both the field and lab, the development and survival of OE-infected and OE-uninfected monarch butterflies, reared in either a warmed or ambient environment, was used to build population projection models to assess changes in monarch fitness due to differing abiotic and biotic conditions. Here, I hypothesized that the physiological stress monarchs experience from both increased temperatures and concomitant parasite

infection would negatively affect population growth, overall dynamics, and decrease aspects of monarch fitness.

In Chapter 4, I designed a course-based undergraduate research experience (CURE) using the monarch butterfly (*Danaus plexippus*) and milkweed (*Asclepias*) system, focusing on an invasive milkweed, *Asclepias curassavica*. Here, I investigated how does the invasive milkweed, *A. curassavica*, compete with two native milkweed species, *A. incarnata* and *A. tuberosa*. Furthermore, I assessed how CURE participation improved student understanding of an ecologically relevant topic (i.e., invasive species biology) as compared to students enrolled in a traditional ecology laboratory by using a hands-on, competition experiment. The factorial competition experiment was carried out over three semesters by CURE students, assessing competition between the invasive *A. curassavica* and two native milkweed species, *A. incarnata* and *A. tuberosa*. I hypothesized that A. curassavica would be a more robust milkweed species across fitness metrics and act as an antagonistic competitor to native milkweed species. Additionally, I hypothesized that early-division CURE laboratory students would display a greater understanding of the ecology of invasive species than upper-division students enrolled in a traditionally structured ecology laboratory.

Finally, in Chapter 5, I summarize and synthesize the major findings of my dissertation. I also discuss the implications on my work on best management practices for monarch butterflies and best gardening practices for milkweed. I conclude my dissertation by briefly outlining my future research directions.

CHAPTER 2
CLIMATE CHANGE AND AN INVASIVE, TROPICAL MILKWEED: AN ECOLOGICAL TRAP FOR MONARCH BUTTERFLIES (*DANAUS PLEXIPPUS*)

INTRODUCTION

As global temperatures continue to rise, species may respond to climate change in a variety of ways. For instance, species may shift their distributions by migrating to unaffected or climatically similar areas (Parmesan and Yohe 2003, Moritz *et al.* 2008). Alternatively, species may undergo phenotypic change that ameliorates negative climate-induced impacts or takes advantage of potential positive effects (i.e., increase in population growth at higher latitudes) (Schlaepfer *et al.* 2002, Deutsch *et al.* 2008, Angilletta 2009). Regardless of the mechanism, climate change research has often focused on the responses of single species to changes in global climate. While this research provides valuable insight into the effects of global warming on generalist consumers, the impacts of climate change on dietary specialists are not as readily apparent (Gough *et al.* 2015). Thus, it has become increasingly recognized that species interactions, especially interactions between tightly-linked species, need to be considered when trying to understand the full impacts of climate change on ecological dynamics (O'Connor *et al.* 2012, Urban *et al.* 2013, Elderd and Reilly 2014).

Whenever rapid environmental change reduces the quality of an organism's

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habitat, including the quality of its diet, there is potential for the species to be caught in an ecological trap (Schlaepfer *et al.* 2002, Battin 2004). Ecological traps occur when organisms make maladaptive habitat choices and/or experience negative phenotypic responses based on environmental cues that once correlated positively with habitat quality and/or evolutionarily stable phenotypic traits (Schlaepfer *et al.* 2002, Robertson and Hutto 2006). In an altered environment, formerly reliable signals may no longer correspond to positive adaptive outcomes and the organism becomes "trapped" by their responses. This may result in a decline in fitness (Schlaepfer *et al.* 2002, Van Dyck *et al.* 2015). Ecological traps due to anthropogenic actions have become increasingly prevalent. For example, off the coast of Western Africa, climate-change induced environmental variability and overfishing have created cool, chlorophyll dense waters, usually indicative of healthy fish populations, that are devoid of fish (Sherley *et al.* 2017). This has created an ecological trap for endangered African penguins which use chlorophyll density as an indicator of good fishing grounds (Sherley *et al.* 2017). However, effects of climate change on species interactions that generate ecological traps represent a recognized but surprisingly little-studied problem (Urban *et al.* 2013). For herbivores, and particularly specialists, rapid changes in the quality of their plant hosts under environmental change may generate ecological traps if the plants upon which they rely become unsuitable.

Many specialists feed either on a single plant species or multiple species within a single genus, and an herbivore's fitness may vary depending upon the type of species and quality of the species being consumed (Ali and Agrawal 2012). For instance, the monarch butterfly (*Danaus plexippus*) feeds almost exclusively on milkweed species within the

genus *Asclepias*. *Asclepias* species vary widely in their production of cardenolides, secondary chemical defenses that the monarch sequesters as an anti-predator *(*Brower *et al.* 1967) or an anti-parasite defense (de Roode *et al.* 2008). Furthermore, *Asclepias* species differ in latex production (Agrawal and Konno 2009), physical defenses, leaf morphology (Agrawal et al. 2009a), and phenologies (Woodson 1954). Individual monarch fitness varies non-linearly with cardenolide production, where more toxic milkweeds confer a greater defense against predators, but can be too toxic to monarchs at high of concentrations, such that intermediate levels result in higher fitness (Malcolm 1994, Sternberg *et al.* 2012). Consequently, any changes, either positive or negative, to milkweed chemistry due to global warming could have corresponding indirect effects on monarch performance.

Even if plant quality is unaffected by increased temperatures, monarch physiology, development, and cardenolide metabolism may change with different temperatures. Monarch larvae exposed to constant, elevated temperatures experience increased mortality, longer developmental times, and weigh less as adults (York and Oberhauser 2002). Additionally, survival and development rates of monarch larvae are maximized at temperatures around 29^{o}C (Zalucki 1982), and increasing temperatures decrease monarch time to pupation (Lemoine *et al.* 2015). While these studies help us to understand the impacts of different temperature regimes on monarch development, little research has been conducted to examine temperature-mediated effects on resource quality. To quantify the potential indirect effects of climate change on herbivore fitness and to gauge whether a warmer planet will result in the creation of an ecological trap, we focused on

the interaction between monarch butterflies (*Danaus plexippus*) and two of their milkweed host plants, *Asclepias curassavica* and *Asclepias incarnata*.

A. curassavica is an exotic, commercially-planted milkweed species found predominantly in the southeastern United States that can negatively affect monarchs by providing a year-round source of food, reducing the propensity to migrate, and thereby increasing disease prevalence in non-migratory populations (Satterfield *et al.* 2015). A majority of monarchs that do not overwinter in Mexico do so in the southern United States (Howard *et al.* 2010), and southern females prefer to reproduce on *A. curassavica* in the fall (Batalden and Oberhauser 2015). In recent years, monarchs have established year-round populations on introduced, invasive *A. curassavica* in the southern United States, potentially to their detriment (Satterfield *et al.* 2015). In contrast, *A. incarnata* is a common, native milkweed species found throughout the eastern and south-eastern portion of the monarch migratory range that senesces during the winter months (Ladner and Altizer 2005, Agrawal et al. 2009a).

If *A. curassavica* quality were to improve due to environmental change, populations of sedentary, non-migratory monarchs could increase. But, if the quality of *A. curassavica* foliage were to decline under environmental change, sedentary monarch populations could fall into an ecological trap. Here, we investigated whether increased temperatures will negatively or positively affect the foliar quality of *A. curassavica* and *A. incarnata* and, subsequently, impact monarch fitness. Because relative differences in host quality can generate ecological traps, comparing our results with those from *A. incarnata*

allows us to show that the invasive *A. curassavica* represents a potential ecological trap under warmer climatic conditions.

MATERIAL AND METHODS

STUDY SYSTEM

Monarch butterflies have a wide distributional range across North America, spanning from central Canada south through central Mexico, with isolated island populations in the Caribbean and Hawaii (Altizer and Davis 2010). Most eastern United States monarch butterflies make an annual, multi-generational migration spanning 3500 km between breeding grounds and overwintering sites (Brower and Malcolm 1991), although sedentary populations have established on *A. curassavica* in Florida, Texas, and Louisiana (Satterfield *et al.* 2015). For our experiment, the monarchs used were from the non-inbred F2 generation of lab reared butterflies. Parent monarchs were collected in Baton Rouge, LA and Katy, TX, USA from migratory monarch populations. Their offspring (the F1 generation) were reared on *A. tuberosa* to ensure F2 offspring naivety to the two focal experimental species, *A. curassavica* and *A. incarnata*. Offspring from the F2 generation were from a single parental pair. Unless infected with parasites, ovipositing monarch females and monarch larvae show no preference between these two milkweed species (Lefèvre *et al.* 2010). Furthermore, monarchs in this study were uninfected with the parasite protozoan parasite, *Ophryocystis elektroscirrha* (OE), based on methods described in Altizer and Oberhauser (1999) and Altizer (2001).

To protect against herbivory, milkweeds have a variety of defensive mechanisms, including latex exudation and production of toxic cardiac glycosides (cardenolides). Latex

is a sticky, viscous substance that is exuded upon tissue damage and can trap early instar monarchs and gum-up larval mouth parts (Agrawal *et al.* 2009b). *A. incarnata* exudes slightly more latex than *A. curassavica* on average (Agrawal and Konno 2009). Cardenolides are toxic steroidal compounds that disrupt the Na+/K+ ATPase system in cell membranes (Malcolm 1991, Bingham and Agrawal 2010). *A. curassavica* is known to have total cardenolide concentrations 11-times higher than those in *A. incarnata*, and *A. curassavica* also contains a much larger number of chemically distinct cardenolides than *A. incarnata* (de Roode *et al.* 2008). Although monarchs sequester cardenolides for their own defense, particularly high cardenolides concentrations can impose significant fitness costs (Zalucki *et al.* 2001, Sternberg *et al.* 2012, Tao *et al.* 2016).

For the experiments described below, all milkweed plants were grown from seeds retrieved from the USDA-NPGS (National Plant Germplasm System). Milkweed seedlings were grown in environmental growth chambers (Conviron CMP6010) set at 16-hr photo-periods at 28°C. The seeds were sown in a mixture of SunGro professional growing soil (www.sungro.com), vermiculite, and Scotts 14-14-14 osmocote fertilizer (www.scotts.com). At the time of the experiment, the individual milkweed plants were 4 months old.

Experimental Design

We conducted a fully factorial experiment to examine how increased temperature and milkweed species identity affect monarch growth and development. We crossed ambient versus elevated temperature with the two milkweed species (*A. incarnata* and *A. curassavica*), and we established ten replicates of each of the four treatments. To warm the experimental sites, we constructed open-top chambers (OTCs) (Godfree *et al.* 2011,

Elderd and Reilly 2014). OTCs were constructed with plexiglass plates (Solar Components Corporation, Manchester, NH, USA) that slant inward to focus solar energy within the plot (Godfree *et al.* 2011). A single, hexagonal OTC consisted of six trapezoidal sections attached with fencing brackets and PVC piping. Each trapezoidal section was supported by a thin, wooden skeleton spanning the outer edges, and was covered by the solar plexiglass. In the center of each plot, we planted a single potted milkweed, which was covered with a butterfly bag (Fig. A.1). The amount of plant biomass for each species in each plot was approximately the same, as milkweeds used were the same age and size. Plots were spaced approximately 3.5 meters apart. In a subset of the plots, we placed iButtons (Maxim Integrated, San Jose, CA, USA), which recorded temperature and humidity every ten minutes. The iButtons were enclosed in a small mesh bag made of the same material covering the individual plants. The bag containing the iButton was then encased in reflective material and placed approximately 15 cm north of the plant and approximately 15 cm aboveground (Brooks *et al.* 2012). The placement of the iButtons, along with being enclosed in a mesh bag covered in reflective material, minimized the chance that the iButtons were exposed to direct sunlight, which can cause large temperature fluctuations. The iButton data allowed us to determine the extent to which the OTCs raised temperature and humidity in experimental warming plots as compared to control plots. Control plots were left in ambient conditions, uncovered by an OTC. The experiment was conducted at Louisiana State University - Innovation Park (Baton Rouge, LA, USA).

There has been some criticism of the use of OTCs as described above since they only raise temperature during the daylight hours when the sun is shining (Godfree *et al.* 2011). To alleviate this concern, Godfree *et al.* (2011) advocated the use of thermal masses (i.e., water-filled PVC pipes) lining the perimeter within the OTCs to maintain treatment differences during the nighttime. Trial runs using thermal masses indicated they did not help to significantly regulate either temperature or humidity compared to non-thermal mass lined OTCs (Faldyn, unpublished data). This is likely due to the fact that in southern Louisiana, average summer humidity stays consistently high (usually above 80%) compared to Central NSW Australia where the thermal masses were first tested. Thus, the thermal masses had less of a regulatory effect on humidity and subsequently temperature.

To acclimatize the plants, plants were placed in their appropriate temperature treatments for 72 hours prior to the beginning of the experiment. After 72 hours, 80 first-instar monarchs (the F2 generation from the lab reared colony), were placed on the plants, sealed within the insect-mesh bag, and allowed to feed normally. Plants were watered every morning and checked daily. After two weeks in the field, all surviving monarchs had pupated. The developing monarchs had adequate plant tissue to support their development to pupation, given that plants had remaining leaf tissue at the end of the experiment. Pupae were brought into the lab once they were observed in the field. Collected pupae were then weighed, sexed, allowed to eclose, and the fate of each larva recorded (i.e., whether or not it survived from 1st instar to adulthood). Adult monarchs

were weighed one day after eclosion (wet weight), sexed, and their forewings measured following (Van Hook *et al.* 2012).

Plant Trait Measurements

To measure plant traits that may be affected by warming, we collected data before and after placing monarch larvae within each of the plots. After the 72-hour acclimatization period, initial samples for carbon, nitrogen, latex, and cardenolide measurements were taken by either measuring the trait in the field (latex) or collecting leaf tissue for subsequent analysis. Once the experiment was concluded and all pupae returned to the lab, we completed a second set of measurements to quantify chemical changes in the host plants.

Milkweed foliar carbon and nitrogen concentrations were analyzed on a Leco TruSpec CN analyzer (http://www.leco.com) and reported in ppm (equivalent to mg/kg of plant samples). Milkweed latex measurements were collected following methods similar to (Agrawal 2005), wherein a fully expanded, intact leaf was clipped (0.5 cm) and the exuding latex was collected on a dried, preweighed 1-cm disk of filter paper, then placed and sealed inside a dried, preweighed Eppendorf vial. The vial was promptly weighed in the lab, and the resulting difference in weight was the "wet" latex weight. The vial was opened and dried overnight at 60°C, and weighed again to collect a "dry" latex weight. Milkweed foliar cardenolide concentrations were quantified using methods modified from Malcolm and Zalucki (1996) and described by Zehnder and Hunter (2007). Leaf tissue was frozen in liquid N_2, and stored in an UltraCold (-80°C) freezer. Leaf tissue was dried, ground using a mini-ball mill, weighed, and then extracted in 100% methanol.

The supernatant from the samples in methanol was vacufuged at 45°C until dry. Samples were then resuspended in either 150 µL of methanol or 75 µL of methanol depending on the dry weight of plant tissue available (dry weight less than 20 mg was resuspended in 75 µL of methanol). Samples were spiked with 0.15 mg/mL digitoxin as an internal standard and analyzed using reverse phase high-performance liquid chromatography (UPLC, Waters Inc., Milford, MA, USA). Running time for each sample was approximately 8 min. Peaks were detected by absorption at 218 nm using a diode array detector, and absorbance spectra were recorded from 200 to 300 nm. Peaks with symmetrical absorption maxima between 217 and 222 nm were recorded as cardenolides. Total cardenolide concentration was calculated as the sum of all separated cardenolide peaks, corrected by the concentration of the internal standard (digitoxin) and the estimated sample mass.

STATISTICAL ANALYSIS

Open-top Chambers & Monarchs

The effects of OTCs on plot temperatures were analyzed using a repeated measures ANOVA across days. Temperature measurements were recorded every 10-minutes, with daytime temperatures averaged between 8am and 8pm, and nighttime temperatures averaged between 8pm and 8am. A base-10 log-transformation was applied to ensure normality. Both daytime and nighttime average temperatures were analyzed to assess OTC performance. Monarch pupal weights, adult weights, and adult forewing lengths were analyzed using a three-way ANOVA between *A. curassavica* and *A. incarnata* host plants, ambient or warmed plots, and monarch sex. Monarch survivorship

was analyzed using a chi-squared analysis between *A. curassavica* and *A. incarnata* host plants and ambient or warmed plots. The repeated-measures ANOVAs, three-way ANOVAs, and chi-squared analysis for the OTCs, monarch, and milkweed data were conducted in SAS 9.4 using the proc mixed and proc freq procedure (SAS Institute Inc 2013). All data were tested to ensure normality.

Milkweed

Milkweed latex exudation, plant carbon:nitrogen ratios, and total cardenolide concentration were analyzed using a repeated measures ANOVA comparing initial (pre-treatment) and final (post-treatment) milkweed tissue. To ensure normality, carbon:nitrogen ratios were base-10 log-transformed and total cardenolide concentrations were square-root transformed. Milkweed cardenolide composition (relative abundance of different molecular types) was analyzed using a permutional MANOVA performed in R using 'adonis' in the 'Vegan' package (Oksanen *et al.* 2015). This acts as an analysis of variance by partitioning among sources of variation and fitting linear models to calculated distance matrices based on these partitions (Oksanen *et al.* 2015). To assess differences in the cardenolide composition of the milkweed, we used metaMDS in 'Vegan' for Nonmetric Multidimensional Scaling (NMDS) (McCune and Grace 2002) with 999 permutations per model run and a maximum of 20 runs per dimension. Model stress declined rapidly from a one-dimensional to a two-dimensional model, declining only slightly thereafter in a three-dimensional model. Model stress is a goodness of fit statistic for the observations, defined so that the sum of squared values is equal to squared stress where large stress values indicate a poor model fit (e.g., stress value between 0.5-0.15 is a

fair fit) (Oksanen *et al.* 2015). We therefore used a two-dimensional model (model stress = 0.1063083), indicating a good ordination fit. We used the NMDS coordinates from this analysis to plot the position of the milkweed cardenolides in multidimensional space.

RESULTS

OPEN TOP CHAMBERS (OTCS)

Overall, the OTCs significantly raised temperatures in the experimental plots ($F_{1,56}$=636.02, p<0.0001). During the daytime, temperatures in the OTC enclosed plots were raised by 3°C, maintaining an average temperature around 35°C, compared to ambient plots with an average temperature of 32°C ($F_{1,28}$=576.12, p<0.0001, Fig. A.2). In daytime hours, monarchs in the OTC plots experienced brief peaks in temperature up to a maximum of 46°C, and in open, ambient plots monarchs experienced temperature peaks of up to 38°C. Nighttime ambient temperatures were lower than nighttime OTC plot temperatures ($F_{1,28}$=60.98, p<0.0001), with an average temperature of 23°C. On average, nighttime temperatures were raised by roughly 0.2°C in OTC covered plots. Additionally, there were significant differences between daytime and nighttime temperatures ($F_{1,56}$=39,170.4, p<0.0001), differences across experimental days ($F_{13,56}$=39,175.22, p<0.0001), and an interaction between experimental day and OTC applications ($F_{1,56}$=441.10, p<0.0001). In general, the increase in temperature in our experimental plots reflects the projected increase in temperature expected at our site by 2080 (Karl *et al.* 2009).

MONARCH

Warmer temperatures had strikingly different effects on monarch survival to adulthood depending on host plant. Specifically, survivorship was five times lower on *A. curassavica*

at warmer temperatures than on *A. curassavica* at ambient temperatures, whereas no differences were seen in monarch survivorship on *A. incarnata* between temperatures (species by temperature interaction, χ_1^2=4.38, p=0.0363, Fig. 2.1A). As expected, pupal weights varied significantly with gender, with male pupae weighing 16% more than female pupae ($F_{1,30}$=6.77, p=0.0143). Marginally significant differences in adult monarch weight were driven by the interaction between the host milkweed plant species and the temperature treatment ($F_{1,23}$=3.07, p=0.0929, Fig. 2.1B), with no observed differences in adult weight between sexes. Adult monarchs forewing lengths decreased by 2.5mm, on average, when exposed to warmer temperatures ($F_{1,20}$=11.4, p=0.003, Fig. 2.1C), with male monarchs having marginally longer forewings overall ($F_{1,20}$=3.99, p=0.0594).

MILKWEED

Across all temperature treatments, the introduced *A. curassavica* exuded more than three times the amount of latex produced by the native *A. incarnata* ($F_{1,37.9}$=43.05, p<0.0001, Fig. 2.2A). After two weeks in the field, both plant species produced more latex by an average of 70% ($F_{1,38.2}$=10.53, p=0.0024). There was no significant main or interaction effect of warming on latex exudation in this experiment. *A. incarnata* had a foliar C:N ratio that was 14% higher than *A. curassavica* ($F_{1,59}$=8.22, p=.0057, Fig. 2.2B), while foliar C:N ratios declined by 13% in both species over the two-week period ($F_{1,59}$=8.7, p=0.0045, Fig. 2.2B).

On average, *A. curassavica* produced 13-fold higher foliar cardenolide concentrations than *A. incarnata* ($F_{1,39}$=299.41, p<0.0001, Fig. 2.2C). Foliar cardenolide concentrations more than doubled in both species over time ($F_{1,39.1}$=25.94, p<0.0001, Fig.

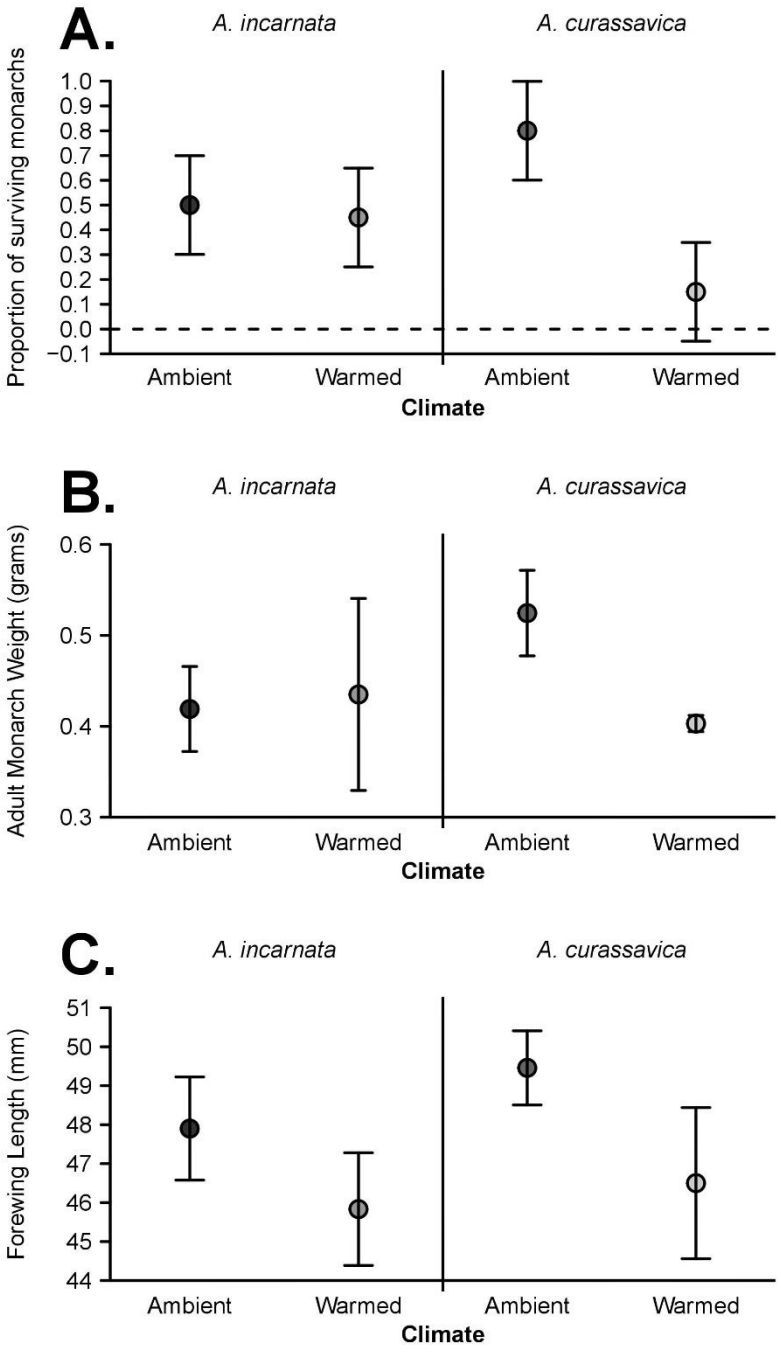

Figure 2.1. The survival (A.), adult mass (B.), and forewing length (C.) of monarch butterflies reared on two milkweed species under ambient and elevated temperatures. (A.) The proportion of surviving adult monarchs, with 95% confidence intervals. Note the significant interaction between the warming treatment and milkweed species. (B.) Average adult monarch weight, with 95% confidence intervals; follows the same significant patterns as the survivorship results. (C.) Average forewing length, with 95% confidence intervals. Note the significant effect of the warming treatment. Darker colors indicate the ambient treatment, while lighter colors indicate the warmed treatment.

2.2C). Importantly, the temporal increases in foliar cardenolide concentrations in *A. curassavica* were higher in the warming treatment, reaching 4 mg/g dry mass ($F_{1,39.1}$=13.02, p=0.0009, Fig. 2.2C). *A. curassavica* produced a 5-times greater variety of cardenolides than did *A. incarnata* (PerMANOVA, $F_{1,55}$=28.7645, p=0.001), with cardenolide composition changing significantly over time (PerMANOVA, $F_{1,55}$=21.7170, p=0.001). The temporal changes in cardenolide composition were more variable among individual *A. incarnata* plants than among individual *A. curassavica* (PerMANOVA interaction, $F_{1,55}$=12.9588, p=0.001, Fig. A.3). Temperature treatment had no effect on milkweed cardenolide composition (PerMANOVA, $F_{1,55}$=1.0704, p=0.349).

DISCUSSION

The exotic, invasive *A. curassavica* represents a potential ecological trap for monarchs given their markedly reduced performance under warmer conditions as compared to current conditions (Fig. 2.1). The dramatic drop in performance may have been driven by increases in total cardenolide production, especially in combination with increased temperatures (Fig. 2.2). Interestingly, this pattern was not driven by changes in the chemical composition of the cardenolides, as the two milkweed species have distinctive profiles (Fig. A.3). Temperature alone did not influence cardenolide composition in either milkweed species (Fig. A.3). We suspect that monarchs performed better on *A. curassavica* than on *A. incarnata* under ambient conditions because the latter has lower foliar N concentrations (Fig. 2.2B). However, the substantial increase in foliar cardenolide concentrations in *A. curassavica* under warming temperatures (Fig. 2.2C) may cause the dramatic decline in monarch performance illustrated in Fig. 2.1. Beyond

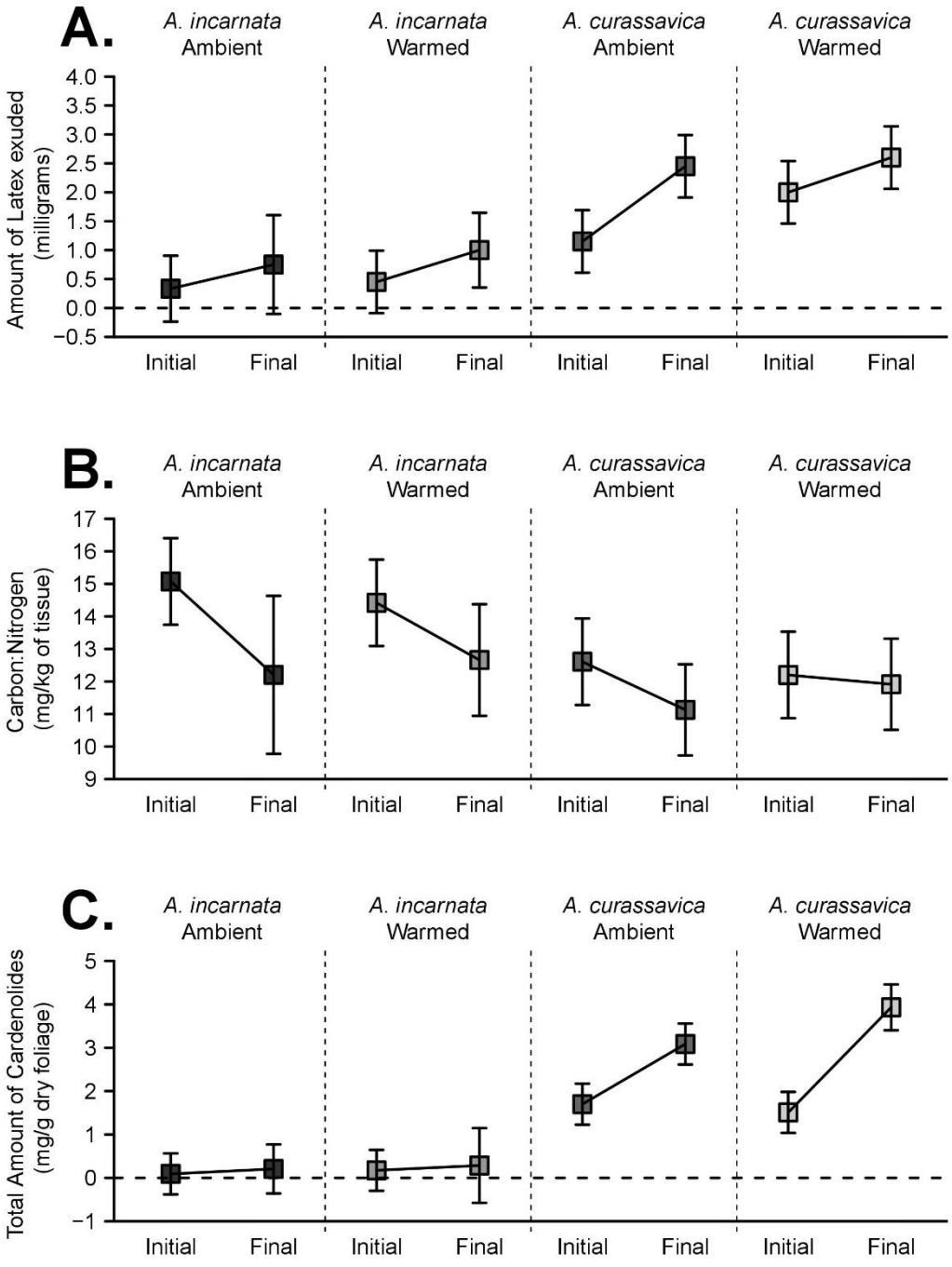

Figure 2.2. Indices of foliar quality of milkweeds grown under ambient and elevated temperatures measured before (initial) and after (final) hosting monarch caterpillars. (A.) The average amount of latex exuded prior to and at the conclusion of the experiment for each experimental treatment with 95% confidence intervals. (B.) The average carbon:nitrogen ratios with 95% confidence intervals. (C.) The average total cardenolide concentration with 95% confidence intervals. Darker colors indicate the ambient treatment, while lighter colors indicate the warmed treatment.

temperature effects on monarchs mediated by diet quality, increased temperatures also decreased monarch forewing lengths (Fig. 2.1C), which may negatively impact monarch flight potential. Alterations in forewing lengths can change wing loading, affecting butterfly flap-glide efficiency, flight speed, and maneuverability (Betts and Wootton 1988). Previous work has noted substantial declines in monarch fitness as cardenolide concentrations approach 3mg/g dry mass (Sternberg *et al.* 2012, Tao *et al.* 2016). Here, by the end of our experiment, foliar cardenolide concentrations exceeded 4mg/g dry mass in *A. curassavica*. Given that neither monarch larvae nor parasite-free, ovipositing female adults appear to choose among milkweed species based on cardenolide concentration (Lefèvre *et al.* 2010, Lefevre *et al.* 2012), warming temperatures may cause *A. curassavica* to function as an ecological trap.

Interestingly, while there were temperature-induced changes in the overall production of defensive compounds by *A. curassavica*, elevated temperatures did not influence the types of compounds produced given that each milkweed species produces a distinctive cardenolide signal (Fig. A.3). Because all experimental bags had larvae within them, we cannot determine whether temporal changes in foliar quality (Fig. 2.2) resulted from ontogenetic change in milkweeds or from induction via herbivory. Previous trials exposing *A. curassavica* and *A. incarnata* to ambient and warmed environments without herbivory have shown that latex exudation decreases with increased temperatures (Faldyn, unpublished data), in contrast to the results reported here in which temperature had no effect on latex exudation. Furthermore, previous studies in other systems have shown that plants with inducible defenses often experience a decrease in inducibility

when exposed to increased temperatures (Zhu *et al.* 2010, DeLucia *et al.* 2012). For *Asclepias*, warmer environmental conditions may lead to increased transpiration, which affects cellular turgor pressure, subsequently impacting latex production as latex exudation is dependent on turgor pressure (Agrawal and Konno 2009). Whether the result of induction or ontogeny, it is clear that milkweed total cardenolide production reaches deleterious concentrations in *A. curassavica* foliage when plants and larvae are reared under warmer temperatures.

Our work explores how temperature influences the interaction between monarchs and milkweeds and compliments previous work that considered independent effects of temperature on monarch development and on milkweed distributions. For example, projected climate change may force breeding niches for monarch butterflies northward (Batalden *et al.* 2007), and current winter range may become inadequate for monarchs due to increased cool weather precipitation (Oberhauser and Peterson 2003). Furthermore, predicted northward shifts of *Asclepias* sp. into Canada may lead to northward shifts in monarch summer distributions (Lemoine 2015). Understanding changes in host plant distributions for tightly-coupled, insect-plant interactions (e.g., the monarch-milkweed system) is crucial, but understanding changes in host resource quality is equally important to consider. Other environmental drivers may also influence these interactions, including water availability (Andrews and Hunter 2015), nutrient deposition (Zehnder and Hunter 2008, Tao *et al.* 2014), and elevated atmospheric concentrations of carbon dioxide (Vannette and Hunter 2014). Biotic interactions with other species may also need to be considered. For example, *A. curassavica* may delay or eliminate migration

due to the year round availability of leaf tissue, and loss of migration increases the monarch's exposure to the protozoan parasite, *Ophryocystis elektroscirrha* (OE) (Satterfield *et al.* 2015). Additional pathogens can interact in complex ways with OE infections, potentially affecting monarch performance more than temperature increases alone (Nifosi and Hunter 2015). While temperature induced changes in milkweed chemistry may benefit monarchs by decreasing parasite loads, it seems unlikely that they could compensate for the dramatic declines in monarch performance illustrated in Fig. 2.1A. Adding the cascading effects of global climate change and other environmental change to the mix may further complicate these interactions.

While our experimental design addresses monarch performance on two distinct host plants at different temperatures, it does not address host plant selection by ovipositing females. Female monarchs may preferentially oviposit on more toxic milkweed plants to reduce parasitic OE virulence in their offspring (Lefèvre *et al.* 2010). Furthermore, in mixed groups of *A. curassavica* and *A. incarnata*, female monarchs selectively oviposit on *A. curassavica* so their offspring can sequester more potent cardenolides (Malcolm and Brower 1986). As some milkweed species increase in total cardenolide concentrations with increasing temperatures, monarchs may oviposit on more potent milkweed that will help medicate against OE infections and improve sequestered defenses. Our experiments may have imposed a substantial stress on milkweeds, potentially inducing changes in foliar quality different from those that may accompany more gradual climate change. However, in addition to increases in average annual temperature, climate models predict concomitant increases in climatic variability,

including a higher frequency of heat waves (Karl *et al.* 2009). Higher annual temperatures and more frequent heat waves may combine to intensify the ecological trap that results from elevated cardenolide concentrations in *A. curassavica*. Ultimately, the combination of direct and indirect effects of multiple drivers will determine the overall effects of environmental change on monarchs and their milkweed hosts. Nonetheless, warming alone appears sufficient to generate an ecological trap for the populations of monarchs feeding on *A. curassavica*.

In general, research continues to show the importance of indirect effects in determining how species respond to climate change (O'Connor *et al.* 2012, Elderd and Reilly 2014, Cerrato *et al.* 2016). The direction and the strength of such interactions may have important fitness consequences regardless of whether or not individual species are consigned to an ecological trap. However, there is generally a temperature optimum at which individual fitness is maximized (Angilletta 2009). If that optimum is surpassed as the Earth warms (Deutsch *et al.* 2008), the species may eventually fall into a trap. Given current trends in planting of *A. curassavica* to alleviate habitat loss, best gardening practices should be reevaluated to reinforce the notion that native milkweed species should be preferentially planted. Additionally, nurseries should work to increase the number of locally native milkweed species sold and work to deemphasize the selling of *A. curassavica*. Overall, we have shown the importance of examining how species interactions may respond to abiotic changes due to climatic drivers. This is particularly true for specialists and their response to global warming. Without gaining proper insight

into how these interactions shift as the planet warms, we may be unwittingly setting ecological traps.

CHAPTER 3
CLIMATE CHANGE AND PARASITE INFECTION, A ONE-TWO PUNCH: THE EFFECTS OF CLIMATE CHANGE AND PARASITE INFECTION (*OPHRYOCYSTIS ELEKTROSCIRRHA*) ON MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*) POPULATION DYNAMICS

INTRODUCTION

Climate change, especially increasing global temperatures, may directly impact species population dynamics by altering their interactions (Karl *et al.* 2009, Post *et al.* 2009). Species interactions provide the structural backbone for ecological communities and are constrained by abiotic and biotic factors (Dunson and Travis 1991). For example, changes in temperature, precipitation, atmospheric CO_2, and pH alter species phenologies, interaction strengths, and individual development (Parmesan 2006, Karl *et al.* 2009). Furthermore, climate change has already altered biotic interactions between species; having caused species-specific range expansions and contractions (i.e., in polar and montane species), while differentially disrupting predator-prey and insect-herbivore interactions (Parmesan 2006, Tylianakis *et al.* 2008). Thus, species interactions, specifically interactions that impact a species population growth, overall life-history, and vital demographic rates, must be considered when assessing the full impacts climate change will have on ecological processes (Gilman *et al.* 2010).

Biotic interactions can differentially affect species responses to climate change, dependent upon the direct impacts climate change poses to a focal species, an interacting species, the interactions themselves, both species dispersal abilities, and the surrounding community structure (Gilman *et al.* 2010). Understanding the role that abiotic factors (e.g., increasing temperature) and biotic factors (e.g., species interactions) have on

population dynamics has grown in both importance and urgency as climate change is affecting species distributions (Altizer *et al.* 2011), population dynamics (Post *et al.* 2009), and community structure (Parmesan and Yohe 2003). For example, host-parasite interactions, an important biotic pressure that structures ecological communities and influences species population dynamics, may be altered by climate change (Dobson and Hudson 1986, Altizer *et al.* 2013, Cizauskas *et al.* 2017). Host-parasite interactions are expected to be impacted by climactic variation through altered host behavior, contact rates with infective stages, births, deaths, and host immune defenses (Altizer *et al.* 2006, Rohr *et al.* 2011). Yet, the impacts of climate change on most wildlife parasites, and thus, their potential interactions with and impact on the population dynamics of hosts, are by and large, empirically untested (Cizauskas *et al.* 2017).

One way to assess the population growth of a species is through population-projection matrix models (Lefkovitch 1965). Population projection models predict a species long-term population growth rates, transient (i.e., short term, non-asymptotic) population dynamics, and extinction probabilities through time and can tease apart how specific demographic rates affect a species population growth (Morris *et al.* 1999, Caswell 2001, Koons *et al.* 2005). By better understanding a species long-term population dynamics, better informed predictions of future population trends and improved conservation efforts can be made (Beissinger and Westphal 1998, Morris *et al.* 1999, Caswell 2001). For example, population projection models for loggerhead sea turtles (*Caretta caretta*) have identified that stronger protection efforts should focus on juvenile turtles, rather than solely on turtle nests (Crouse *et al.* 1987). Additionally, population

projection models have been used extensively in fisheries sciences to assess fishery yields and productivity (Schaefer 1954). Thus, projection models assessing the population dynamics of a species will be sensitive to the complex interplay of abiotic factors (e.g., changes in temperature) and biotic pressures (e.g., parasitism) (Tylianakis *et al.* 2008).

Specialist species, or tightly-linked interactions between a host and consumer wherein a consumer feeds on a single species or genus and that consumer's fitness depends on the host type and quality, act as small-scale, community modules that can elucidate interactions that often occur at the community level (Holt and Polis 1997, Ali and Agrawal 2012). These tightly-linked, specialist interactions are often seen between phytophagous insects and their parasites. Thus, these host-parasite interactions make excellent models with which to study the effects of climate change on population dynamics. The charismatic monarch butterfly (*Danaus plexippus*), a specialist of milkweed (*Asclepias* sp.), is already experiencing population declines due to increasing parasite infection from the protozoan parasite, *Ophryocystis elektroscirrha* (OE) (Belsky and Joshi 2018). For example, *Ophryocystis elektroscirrha* spores can be easily transmitted, passing vertically from infected males or females and horizontally via larval exposure to OE-infected adults (Altizer *et al.* 2004). Apart from stresses due to parasite infection, elevated temperatures increase monarch mortality (Zalucki 1982) and developmental times (Lemoine *et al.* 2015), while decreasing adult monarch weight (York and Oberhauser 2002). Compounding this, an invasive, preferentially-sold milkweed species, *Asclepias curassavica*, undergoes phytochemical changes from increased temperatures, leading to increasing OE prevalence in monarch populations and forming

ecological traps (Satterfield *et al.* 2015, Faldyn *et al.* 2018). While a substantial body of work has described the impacts of different temperature regimes, CO_2 levels (Decker *et al*. 2018, Decker *et al.* 2019), and population sizes on monarch development, host plant interactions, and migratory propensity (Semmens *et al.* 2016, Oberhauser *et al.* 2017), limited empirical research has been conducted to examine how elevated temperatures affect monarch-parasite interactions and how those interactions impact monarch population dynamics and overall fitness.

To address this, we assessed how elevated temperatures and parasite infection affect monarch butterfly population dynamics and overall fitness. We performed field and lab experimentation tracking monarch development throughout their life cycle in either a warmed or ambient environment, while simultaneously assessing how OE-parasite infection altered monarch population dynamics and fitness metrics. I hypothesize that the physiological stress monarchs experience from both increased temperatures and concomitant parasite infection would negatively affect population growth, overall dynamics, and decrease aspects of monarch fitness. This study will help provide clarity for how climate-change induced increases in temperature, along with concomitant parasite infection, affects host species population dynamics and provides a more complete picture on the impacts climate change exerts on ecological communities.

MATERIAL AND METHODS

THE HOST-PARASITE SYSTEM

Monarch butterflies (*Danaus plexippus*) are a well-known insect species because of their use in k-12 education (Matthews *et al.* 1997, Eick 2012), citizen science engagement

(Howard *et al.* 2010), important pollinator status (Brower *et al.* 2006), and for their annual, 3500-km, multi-generational migration (Brower and Malcolm 1991). Yet, monarch populations have experienced historic population declines due to multiple interacting factors such as environmental change, habitat loss, and increasing *Ophryocystis elektroscirrha* infection (Belsky and Joshi 2018).

Ophryocystis elektroscirrha is a neogregarine protozoan parasite with a life-history dependent upon host (i.e., monarch) development (McLaughlin and Myers 1970), where infection reduces monarch survival, eclosion success, wingspan, reproductive success, and overall activity (Altizer and Oberhauser 1999). Since monarchs are dietary specialists, meaning their host plants are mostly milkweed species within the genus *Asclepias* (Malcolm and Brower 1989), and become infected by consuming parasite spores on milkweed tissue, these host-parasite dynamics are modulated through milkweeds (de Roode *et al.* 2008). Dormant *Ophryocystis elektroscirrha* spores, residing on the surface of the egg chorion or on surrounding leaf tissue, are consumed by developing monarchs (McLaughlin and Myers 1970). The parasite develops and replicates in the larval gut, and emergent butterflies are covered in and shed dormant parasite spores (Altizer and Oberhauser 1999). Monarchs sequester toxic compounds from highly-toxic milkweeds to mitigate some of the negative fitness costs associated with parasite infection (Brower *et al.* 1967, Lefèvre *et al.* 2010).

For our experiment, *Ophryocystis elektroscirrha* infected monarchs used were from the non-inbred F2 generation of lab reared butterflies. Parent monarchs were collected in Baton Rouge, LA, USA from migratory monarch populations. Their offspring (the F1

generation) were reared on *A. syriaca*. Offspring from the F2 generation were from a single parental pair. Post-experiment OE-assessment indicated OE-infection (Fig. B.1C). *Ophryocystis elektroscirrha* uninfected monarchs were the non-inbred F2 generation of lab reared butterflies shared from Sonia Altizer's (University of Georgia, Odum School of Ecology, GA, USA) lab reared colony.

EXPERIMENTAL SETUP

We conducted a full factorial experiment in the lab and field to examine how increased temperature and *Ophryocystis elektroscirrha* infection affect monarch population demography and aspects of fitness. The experiment was performed with twenty-five and fifty replicates of each ambient and warmed treatments in the lab and twenty replicates of each ambient and warmed treatments in the field to mimic more natural settings (Fig. B.1A and Fig. B.1B). OE-uninfected manipulations occurred only in laboratory settings, with sixty replicates in each ambient and warmed treatment.

Lab-reared, OE-infected experimental design

The monarchs used here were likely infected with *Ophryocystis elektroscirrha* from their F1 mother and/or father. In the laboratory, seventy-five infected caterpillars were split between multiple CMP6010 environmental chambers, one set to match ambient conditions (14-hr photoperiod at 29.4°C in 67% RH) and two others set to match predicted climactic conditions for 2070 (14-hr photoperiod at 32.5°C in 84% RH) (Fig. B.1A). OE-infected laboratory monarchs were moved between the chambers evenly to account for any chamber effect. Monarchs were reared from egg to adults in their respective temperature treatments for both in the field and lab, while being fed fresh

clippings from common milkweed, *Asclepias syriaca*. Each day, previously undamaged, virgin milkweed plants, reared in a grow-room in 16-hr photoperiod at 28°C, were defoliated so as to avoid the impacts of milkweed chemical induction. The amount of plant biomass given each day was approximately the same, and monarchs were monitored and fed daily. Monarch eggs were allowed to hatch in the lab, then were moved to either the laboratory or field experiments. Monarch larvae had their own mesh bags (field) or containers (lab), and were checked daily for transitioning into the next stage by collecting their head-capsules and molted outer-skin. Field monarchs did not have pictures taken or weights collected to minimize their exposure to non-experimental conditions, but lab-reared monarch pictures and weights were collected. Once all surviving field monarchs pupated, they were brought into the lab to eclose into adult butterflies. Laboratory larvae were weighed as 5th instar caterpillars and photographed (using a Canon PowerShot SD1200 IS 10MP camera) for melanism and size measurements. Pupae were weighed and photographed for melanism and size measurements, then were allowed to eclose in their respective temperature treatments. Infected monarchs in the field had their development monitored and pupae weighed and photographed. Monarch life-history data were collected through daily censusing, classified by life-history transitions, the date of the census, an individual identifier, information on the life stage, and reproductive status (Stubben and Milligan 2007). For the OE-infected experiment, to maximize the number of monarchs used, monarchs only had life-history data collected after hatching. In total, OE-infected monarch butterflies reared in the lab had survival, OE-infection status, development time (e.g. time in each growth stage), melanism (e.g.

pigmentation intensity, etc. for larvae and pupae), and size (e.g. length, dorsal area, and weight for larvae and pupae) assessed. Combined, these metrics approximate differentially highlight aspects of monarch fitness.

Field-reared, OE-infected experimental design

In the experimental field site, data collection mimicked the laboratory data collection. All field plots contained an insect-proof mesh bag positioned from a wooden stake that held the developing monarch and leaf tissue (Fig. B.1B). To warm the experimental field plots, we constructed open-top chambers (OTCs) (Godfree *et al.* 2011, Elderd and Reilly 2014) described in (Faldyn *et al.* 2018). Briefly, OTCs were constructed with plexiglass plates (Solar Components Corporation, Manchester, NH, USA) that slant inward to focus solar energy within the plot (Godfree *et al.* 2011). A single, hexagonal OTC consists of six trapezoidal sections attached with fencing brackets and PVC piping supporting a wooden skeleton. Plots were spaced approximately 3.5 meters apart. In a subset of the plots, we placed iButtons (Maxim Integrated, San Jose, CA, USA), which recorded temperature and humidity every ten minutes. The iButtons were enclosed in a small mesh bag encased in reflective material and placed approximately 15 cm north of the monarch and approximately 15 cm aboveground (Brooks *et al.* 2012). The placement of the iButtons, along with being enclosed in a mesh bag covered in reflective material, minimized the chance that the iButtons were exposed to direct sunlight, which can cause large temperature fluctuations. The iButton data allowed us to determine the extent to which the OTCs raised temperature and humidity in experimental warming plots as compared to control plots.

There has been some criticism of the use of OTCs as described above since they only raise temperature during the daylight hours when the sun is shining (Godfree *et al.* 2011). To alleviate this concern, Godfree *et al.* (2011) advocated the use of thermal masses (i.e., water-filled PVC pipes) lining the perimeter within the OTCs to maintain treatment differences. Trial runs using thermal masses indicated they did not help to significantly regulate either temperature or humidity compared to non-thermal mass lined OTCs (Faldyn, unpublished data). This is likely due to the fact that in southern Louisiana, average summer humidity stays consistently high (at 80%) compared to Central NSW Australia, where the thermal masses were first tested. Thus, the thermal masses had less of a regulatory effect on humidity and subsequently temperature. Control plots were left in ambient conditions, uncovered by an OTC. The laboratory portion of this experiment was conducted in the LSU Life Sciences Building (Baton Rouge, LA, USA) (Fig. B.1A). The field portion of this experiment was conducted at Louisiana State University - Innovation Park (Baton Rouge, LA, USA) (Fig. B.1B).

The intent was to collect the adult monarchs reared in the field for measurements, but severe and tragic flooding in Baton Rouge, LA, USA (National Weather Service 2016) during the same time impeded campus visits, and tracking adult monarch eclosion and collection of fitness metrics could not be completed. Larval and pupal development, weight, pigmentation, and size was collected. Assessing OE-infection post-experimentation followed methods detailed in (Altizer 2001).

Lab-reared, OE-uninfected experimental design

Ophryocystis elektroscirrha uninfected monarchs were the non-inbred, F2 generation of butterflies shared from Christen Steele at Tulane University, who received them from Sonia Altizer (University of Georgia, Odum School of Ecology, GA, USA). Monarch larva were reared and monitored similarly to the lab reared, OE-infected monarchs previously described. Due to an unexpected milkweed die-off, clippings from swamp milkweed, *Asclepias incarnata*, and from orange-butterfly weed, *Asclepias tuberosa* were used as an additional food source. Any change in food source was applied to all monarchs at the same time, and these three species milkweed, while possessing different morphological and phenological characteristics (Woodson 1954), are all relatively low in cardenolide toxicity (Agrawal *et al.* 2012) and do not affect monarch survivorship or development differentially (Pocius *et al.* 2017). Throughout monarch development, metrics for monarch survival, development time (e.g. time in each growth stage), monarch melanism (e.g. pigmentation intensity, etc. for larvae, pupae, adult forewings, and adult hindwings), and monarch size (e.g. length, dorsal area, and weight for larvae, pupae, and adults) were collected in a similar manner.

Monarch Population Projection Models

The life cycle of holometabolous insects (e.g., monarch butterflies) has four development stages (Gullan and Cranston 2014)(Fig. 3.1):

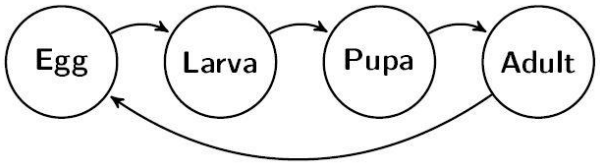

Figure 3.1. The life cycle of the monarch butterfly, a holometabolous lepidopteran insect species. Through metamorphoses, monarchs hatch from an egg into a larva, then into a pupa and eclose as an adult butterfly.

Holometabolous insects can only transition from one stage to the next without reverting to a previous stage, as indicated by the arrows (representing transitions). The life cycle can be further divided into survival, growth, and reproductive elements, with consideration that monarch butterfly larvae go through five distinct larval instar stages (Altizer and Oberhauser 1999)(Fig. 3.2):

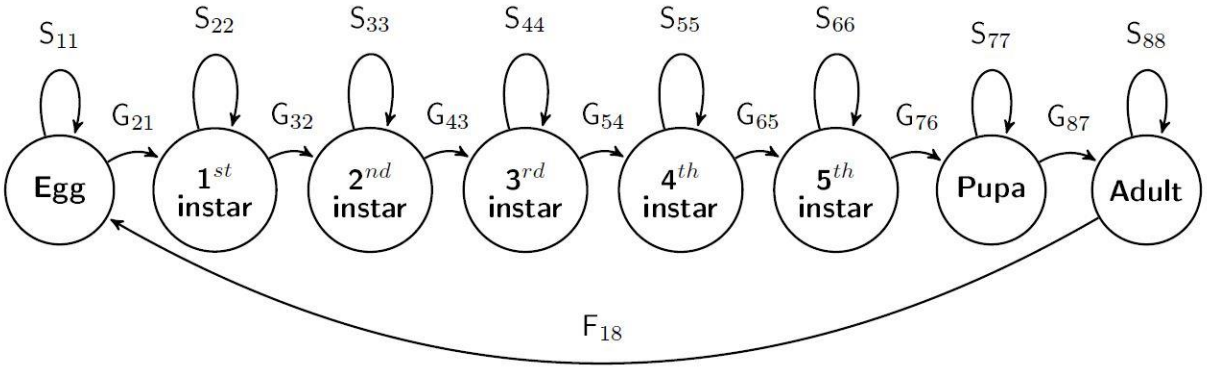

Figure 3.2. The stage-structured life history diagram of the monarch butterfly. Here, the life cycle of hatching from an egg into a larva, then developing into a pupa and eclosing into and adult butterfly remains (Fig. 3.1). But, consideration is given to survivorship in the same age class (S), the probability of growing (G) to a new class, and the fecundity (F), or number of eggs oviposited, by a female monarch.

In this stage-structured demographic model, the arrows between the stages (circled) represent transitions from one developmental stage to the next, indicating the probability of growing (G) from one developmental stage class to the next (horizontal arrows) or the probability of surviving in the same (S) developmental stage (upper curved arrows). The curved arrow at the bottom represents the fecundity (F), or number of eggs oviposited, by a female monarch. A further explanation of each individual vital rate for the monarch stage-structured demographic model can be found in Table B.5. These growth stages can be ordered in a stage-structured matrix, M:

	Egg	1^{st} instar	2^{nd} instar	3^{rd} instar	4^{th} instar	5^{th} instar	Pupa	Adult
Egg	S_{11}	0	0	0	0	0	0	F_{18}
1^{st} instar	G_{21}	S_{22}	0	0	0	0	0	0
2^{nd} instar	0	G_{32}	S_{33}	0	0	0	0	0
3^{rd} instar	0	0	G_{43}	S_{44}	0	0	0	0
4^{th} instar	0	0	0	G_{54}	S_{55}	0	0	0
5^{th} instar	0	0	0	0	G_{65}	S_{66}	0	0
Pupa	0	0	0	0	0	G_{76}	S_{77}	0
Adult	0	0	0	0	0	0	G_{87}	S_{88}

Where the top, horizontal row represents the current stage of development, and the left column refers to a stage one time-step in the future, $(t + 1)$. Each time-step is reflective of one day. Fecundity rates form the top row, and probabilities of either surviving (S) form the diagonal and growing (G) between age classes are in the subdiagonal.

Using matrix (M), the population growth rate and stable stage distribution for monarch butterflies can be calculated using deterministic projections from the dominant eigenvalue and right eigenvector (Caswell 2001), where:

$$n_{t+1} = M \times n_t$$

Describes the population projection from time (t) to time (t+1):

$$[\lambda = A_{nt}] = n_{t+1} = \begin{pmatrix} \text{Egg}_{t+1} \\ \vdots \\ \text{Adult}_{t+1} \end{pmatrix} = \begin{pmatrix} S_{11} & \cdots & F_{18} \\ \vdots & \ddots & \vdots \\ \ddots & \cdots & S_{88} \end{pmatrix} \begin{pmatrix} \text{Egg}_{t} \\ \vdots \\ \text{Adult}_{t} \end{pmatrix}$$

Here, the population is projected from time (t) to time $(t + 1)$ by multiplying matrix (M) by a vector of abundance, (n_t), resulting in a vector of abundances at a future time step, (n_{t+1}). The process is repeated for the next time step with a new vector of abundances, and when repeated over (x) number of time steps, a stable stage distribution (SSD) is reached. Here, (λ_t) remains constant from one time step to the next. This stabilized (λ_t)

is the population growth rate, (λ), or the population size projected into the future for some number of time steps (A_{nt}) (Caswell 2001, Stubben and Milligan 2007).

Sensitivity analysis reveals how very small changes in (S_{ij}), (G_{ij}), or (F_{ij}) affect (λ) when the other elements in the matrix, M, are held constant. Following (Caswell 2001), the sensitivity, s_{ij}, of any vital rate in the matrix (M) is given by:

$$s_{ij} = \frac{v_i w_j}{\langle w, v \rangle}$$

Where v_i is the i^{th} element of the reproductive value vector, w_j, is the j^{th} element of the stable stage vector, and $\langle w, v \rangle$ is the product of the w and v vectors. Thus, the sensitivity of λ to changes in a given vital rate, a_{ij}, is proportional to the product of the i^{th} element of the reproductive value vector and the j^{th} element of the stable stage vector (Caswell 2001). Larger sensitivity values for a given vital rate, a_{ij}, indicate that vital rate a_{ij} has a greater impact on λ.

Vital rates S and G are survival and growth probabilities, whereas fecundity values, F, are not. Therefore, the sensitivity of λ to changes in S and G is difficult to compare with the sensitivities of fecundity, F, rates (de Kroon *et al.* 2000). To address this, elasticity analysis estimates the effect of a proportional change in any given vital rate, a_{ij}, on λ (Caswell 2001) through:

$$e_{ij} = \frac{a_{ij} s_{ij}}{\lambda}$$

Where the elasticity of a matrix element, e_{ij}, is the product of the sensitivity of a matrix element, s_{ij}, and the vital rate itself, a_{ij}, divided by λ (Caswell 2001). Elasticities are proportional, dimensionless sensitivities, allowing for direct comparisons among all vital

rates as they impact λ (de Kroon *et al.* 2000). Ultimately, the biological interpretation of sensitivity and elasticity values is very different. Sensitivities estimate how absolute changes in parameter values affect λ and appropriately quantify the intensity and direction of selection on the importance of life-history transitions and evolutionary questions (de Kroon *et al.* 2000). Thus, including sensitivities with elasticities help predict the results of simultaneous changes in multiple transitions, and can be compared between species with different life histories (de Kroon *et al.* 2000).

Monarch fitness metrics

Ophryocystis elektroscirrha infection was assessed using methods described in (Altizer and Oberhauser 1999, Altizer 2001). Briefly, parasite loads were measured by pressing transparent tape (cut roughly into a 1 cm^2 section) against the ventral side of the monarch abdomen. Based on the number of spores per cm^2, butterflies were scored for parasite loads, scaled as: 0 = no spores, 1 = 1 spore, 2 = 2–20 spores, 3 = 21–100 spores, 4 = 101–1000 spores and 5 = more than 1000 spores (Altizer and Oberhauser 1999). Since monarchs used in the experiment all came from the same family, and OE is passed vertically and horizontally where dormant spores shed from an infected adult are consumed by larvae (Altizer *et al.* 2004), it is likely larvae were initially heavily infected (Fig. B.1C). Survivorship and developmental data were collected as monarchs were checked daily for either successful transitions to the next growth stage or if they died. Larval, pupal, and adult weight were collected prior to images being taken for assessing monarch melanism and size. Methods described in Davis *et al.* (2005) and Schneider *et al.* (2012) were used to assess differences in larval, pupal, and adult monarch melanism and

size. Briefly, images had background pixels removed using Microsoft Word 2016 (Microsoft Corporation 2016). Cropped images were loaded into the image processing software, ImageJ, converted to 8-bit grayscale, and had a pixel scale set to 1-cm in length. A line drawn from the head capsule to the anal prolegs on the larva, the head to the cremaster on the pupa, and across the forewing and hindwings following (Van Hook *et al.* 2012) quantified the length of each stage. Tolerance was set to select the entire larvae, pupae, forewing, or hindwing, and the dorsal area and pigmentation intensity (mean gray color intensity) was collected. Finally, the percent area that is non-white was assessed by converting the image to binary, making a single-pixel outline of the monarch, and quantifying the percentage of pixels that were black in comparison to the overall monarch area.

STATISTICAL ANALYSIS AND MODELING

Open-top Chambers

The effects of OTCs on plot daytime and nighttime temperatures and humidity were analyzed using repeated measures ANOVAs across days through the proc mixed procedure (SAS Institute Inc 2013). Temperature and humidity measurements were recorded every 10-minutes, with daytime averages taken between 8am and 8pm, and nighttime averages collected between 8pm and 8am. A base-10 log-transformation was applied to daytime temperatures to ensure normality. Non-parametric Kruskal-Wallis tests, using the proc npar1way procedure (SAS Institute Inc 2013), were used to analyze differences in nighttime temperatures when normality was not met. Both daytime and nighttime average temperature and humidity were analyzed to assess OTC performance.

Monarch Population Stage-Structured Matrix Modeling

Matrix models were built and analyzed following methods outlined by Stubben and Milligan (2007) using R package 'popbio'. This package was built specifically to facilitate in the construction and analysis of population protection matrix models (Stubben and Milligan 2007). Values for egg survival, S_{11}, and transition to a 1st instar larva, G_{21}, were used from the OE uninfected monarchs. These values are expected to be similar because OE must first be consumed by a newly hatched larva to initiate infection (Altizer *et al.* 2004). Values for egg survival, S_{11}, and transition to a 1st instar larva, G_{21}, occur irrespective of infection as an egg that has not hatched or a successfully hatching monarch cannot be infected by OE until spore consumption. Due to inaccessibility from flooding (National Weather Service 2016), daily censusing for adult monarch survival values (S_{88}) and reproductive, or fecundity, value (F_{18}) had to be estimated. In the OE-infected, warmed, field experiment, the only surviving female eclosed after the flooding to collect a wet weight. Adult survivorship, S_{88}, was estimated using fall-season adult monarchs that migrate from the south to Mexico (S_{20} in Table 1 from Oberhauser *et al.* (2017)). A 6% reduction to adult survival in warm conditions, from Martínez *et al.* (2014), was applied to the value used for S_{88}. From Zalucki (1981), the non-linear, total lifetime fecundity across the daily egg-laying lifespan of monarchs was fit with a 3rd-order polynomial equation. Using this equation and the total lifetime fecundity of monarchs from Zalucki (1981), a daily percentage of total fecundity was calculated. Since campus flooding delayed censusing, and eclosed OE-infected adult females were not able to be immediately weighed after eclosion, lifetime fecundities for monarchs from this

experiment were predicted using wet pupal weight following methods described in Honěk (1993). Lifetime fecundities for OE-infected and OE-uninfected monarchs were incorporated into the matrix models as predicted daily oviposition rates (based on wet pupal weights) across the average, non-linear, egg-laying lifespan for monarch butterflies. Functions 'projection.matrix' and 'pop.projection' were used to build each monarch projection matrix and assess the stable stage distribution for each matrix (Fig. B.4). Function 'eigen.analysis' was used to project each projection matrices growth rate, λ, the vital rate sensitivity values, and vital rate elasticity values. Ultimately, since adult survivorship and fecundity were measured with less precision than juvenile stages, sensitivity and elasticity analyses should elucidate the importance of these vital rates to monarch life-history overall. Finally, across all treatments for each monarch population, stage vector projections indicate expected short-term dynamics and ultimately converge to stable distributions (Fig. B.4).

Monarch Fitness Metrics

Monarch fitness metrics (i.e., development time, monarch melanism, and monarch size across all life stages) were analyzed using a permutational MANOVA performed in R using 'adonis' in the 'Vegan' package (Oksanen *et al.* 2015). To maximize variable input, analyses were performed on monarchs that were reared in laboratory settings, comparing metrics across *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies between ambient and warmed conditions. If *Ophryocystis elektroscirrha* infected monarchs from the field are included, the analysis is limited to metrics only for development time and adult weight. While this highlights a difference between

experimental location (likely driven by a single monarch surviving under warmed field conditions), the overall results of temperature exposure impacting monarch metrics remains unchanged (Fig. B.3). The permutational MANOVA acts as an analysis of variance by partitioning among sources of variation and fitting linear models to calculated distance matrices based on these partitions (Oksanen *et al.* 2015). To assess differences across monarch fitness metrics between temperature treatment and OE-infection, we used metaMDS in 'Vegan' for non-metric multidimensional scaling (NMDS) (McCune and Grace 2002) with 999 permutations per model run and a maximum of 100 runs per dimension. For both OE-infected and OE-uninfected models, model stress declined rapidly from a one-dimensional to a two-dimensional model in roughly twenty runs, declining only slightly thereafter. Model stress is a goodness of fit statistic for the observations, defined so that the sum of squared values is equal to squared stress where large stress values indicate a poor model fit (e.g., stress value between 0.1-0.2 is a good fit) (Oksanen *et al.* 2015). We used a two-dimensional model (model stress = 0.13, indicating a good ordination fit) for comparing differences in OE-infected monarchs, and a two-dimensional model (model stress = 0.16, a good ordination fit) for OE-uninfected monarchs. NMDS coordinates from these analyses were used to plot monarch fitness metrics in multidimensional space. ANOVAs were used to analyze differences in monarch fitness metrics across OE-infected and OE-uninfected butterflies using the proc mixed procedure (SAS Institute Inc 2013). The assumption of normality was violated only for days-in-development data, due to most responses being whole numbers with very limited variation. For a more conservative estimate, non-parametric Kruskal-Wallis tests were

used when normality was not met using the proc nparıway procedure, described by SAS Institute Inc (2013), for the main effects of temperature treatment, experimental location, and sex. For days-in-development data, interactive effects of temperature treatment, experimental location, and sex, parametric results are reported as examining the normal quantile plot approximated normality. Across all comparisons, a sequential bonferroni correction was applied using the proc multtest procedure (Rice 1989, SAS Institute Inc 2013).

RESULTS

OPEN-TOP CHAMBERS

Overall, the OTCs significantly raised temperatures in the experimental plots (Fig. B.2A). During the daytime, temperatures in the OTC enclosed plots were raised by 2.5°C, maintaining an average temperature around 36°C, compared to ambient plots with an average temperature of 33°C ($F_{1,82}$=278.41, p<0.0001, Fig. B.2A). In daytime hours, monarchs in the OTC plots experienced brief peaks in temperature up to a maximum of 43°C, and in open, ambient plots monarchs experienced temperature peaks of up to 38°C (peaks occurring on the same day). Additionally, there were differences across experimental days during the daytime ($F_{11,82}$=117.84, p<0.0001). Nighttime ambient temperatures and nighttime OTC plot temperatures, with an average temperature of 23°C, were not different from one another χ_1^2=1.6, p=0.2061, Fig. B.2A). Furthermore, nighttime-to-nighttime temperatures varied during experimental days (χ_1^2=76.601, p<0.0001). Overall, there was no interaction between OTC application and experimental days on temperature. In general, the increase in temperature in our experimental plots

reflects the projected increase in temperature expected at our experimental site by 2080 (Karl *et al.* 2009).

OTCs marginally raised relative humidity in the experimental plots, specifically during nighttime (Fig. B.2B). During the daytime, OTCs did not increase relative humidity levels compared to ambient plots (Fig. B.2B), although there was significant daytime-to-daytime variation in relative humidity across experimental days ($F_{11,48}$=55.51, p<0.0001). During the daytime, average relative humidity in both the OTC-warmed and ambient plots was roughly 70% (Fig. B.2B). OTCs increased nighttime relative humidity by 1% compared to ambient plots ($F_{11,48}$=5.49, p=0.023, Fig. B.2B), with significant nighttime-to-nighttime variation in relative humidity across experimental days ($F_{11,48}$=6.17, p<0.0001). During the nighttime, average relative humidity in OTC-warmed plots was 95%, and the relative humidity in ambient plots was roughly 97% (Fig. B.2B). There was no interaction between OTC application and experimental days on relative humidity. Overall, relative humidity was consistent between OTC-warmed and ambient plots.

MODELING MONARCH POPULATION DYNAMICS

Warmer temperatures decrease monarch population growth, which is marginally impacted by OE-infection. Across all treatments, population growth rates were highest in ambient conditions, with OE-infection having limited impact on population growth (Table 3.3), yet infection negatively affects several monarch fitness metrics. On average, monarch populations reared under ambient conditions had a 10% higher growth rate than those reared in warmed conditions (Table 3.3). Yet, while warmed conditions depressed

population growth rates overall, OE-infection only decreased population growth in the OTC-warmed, field population and did not decrease population growth in the other treatments (Table 3.3).

Interestingly, sensitivity and elasticity analyses indicated population growth rates were most sensitive to changes in eclosion from pupa to an adult, G_{87}, (Fig. B.5), with pupal survivorship, S_{77}, being the most elastic element across all populations (Fig. B.6), regardless of temperature conditions and infection status (Table 3.3). OE-infected field monarchs, reared in warmer temperatures, were the only exception in regards to sensitivities, as this growth rate was most sensitive to changes in late-instar caterpillar pupation, G_{76} (Table 3.3, Fig. B.5), likely driven by only a single female monarch surviving.

MONARCH FITNESS METRICS

Monarch Survivorship and Infection

Warmer temperatures dramatically reduced monarch survival. For OE-infected monarchs, warmer conditions reduced survival nearly 3.5-times that of monarchs reared under ambient conditions (χ_1^2=19.836, p<0.0001, Fig. 3.4A). An interaction between experimental location and treatment condition indicated that OE-infected monarchs, reared in the lab under warmed conditions experienced reduced survivorship nearly 2-times less than monarchs reared in ambient, lab conditions (Fig. 3.4A). Whereas OE-infected monarchs, reared at the field in OTC-warmed conditions, experienced a 15-fold decrease in survival compared to other monarchs reared at ambient, field conditions ($F_{1,111}$=5.06, p=0.026, Fig. 3.4A). OE-uninfected monarchs showed similar results, in that

Table 3.3. Monarch butterfly vital rates for each population, between experimental location and infection status. Note the decrease in population growth rates for each warmed experiment, and the consistency of the most sensitive vital rate, eclosing into an adult from a pupa (G_{87}), and the most elastic vital rate, surviving as a pupa (S_{77}), across all runs.

OE Infection status	Location	Treatment	Lambda (λ)	Most sensitive matrix element	Sensitivity value	Most elastic matrix element	Elasticity value
Infected	Lab	Ambient	1.173	G_{87}	0.833	S_{77}	0.153
Infected	Lab	Warm	1.088	G_{87}	0.453	S_{77}	0.149
Infected	Field	Ambient	1.197	G_{87}	0.619	S_{77}	0.168
Infected	Field	Warm	1.038	G_{76}	0.704	S_{77}	0.214
Uninfected	Lab	Ambient	1.143	G_{87}	0.412	S_{77}	0.154
Uninfected	Lab	Warm	1.067	G_{87}	0.639	S_{77}	0.29

OE-uninfected, lab reared monarchs at warmer temperatures experienced a 6-fold decrease in survivorship compared to OE-uninfected monarchs at ambient conditions (χ_1^2=34.539, p<0.0001, Fig. 3.4A).

For the OE-infected manipulations, post-experimental OE assessments indicated that monarchs used were indeed OE-infected, but dramatic differences in infection status were observed depending on the environmental conditions. Regardless of being the lab or field, monarchs reared in warm conditions had an OE spore load that was roughly 5-times less than that of monarchs reared at ambient conditions, effectively a OE spore load of zero (χ_1^2=34.561, p<0.0001, Fig. 3.4B), based on the scale described in Altizer (2001). For perspective, monarchs reared in warm conditions had an average OE-spore load of 4.3 (Fig. B.1C), whereas monarchs that survived warm conditions had an average OE-spore load of 0.002 (Fig. B.1D) at the conclusion of the experiment (Fig. 3.4B). OE-infection did not differ between lab and field experiments, and was not disproportionate between male and female monarchs.

OE-Infected Monarch Fitness Metrics

For OE-infected, laboratory monarchs, temperature treatment conditions (either ambient or warmed) dramatically impacted fitness metrics, with differences in monarch sex having little impact (PerMANOVA, $F_{1,30}$=15.58, p=0.001, Fig. 3.5A). Furthermore, while differences across fitness metrics exist between laboratory and field experimental sites (PerMANOVA, $F_{1,46}$=55.95, p=0.001, Fig. B.3), temperature treatment conditions have the greatest impact on differences in monarch fitness metrics overall (PerMANOVA,

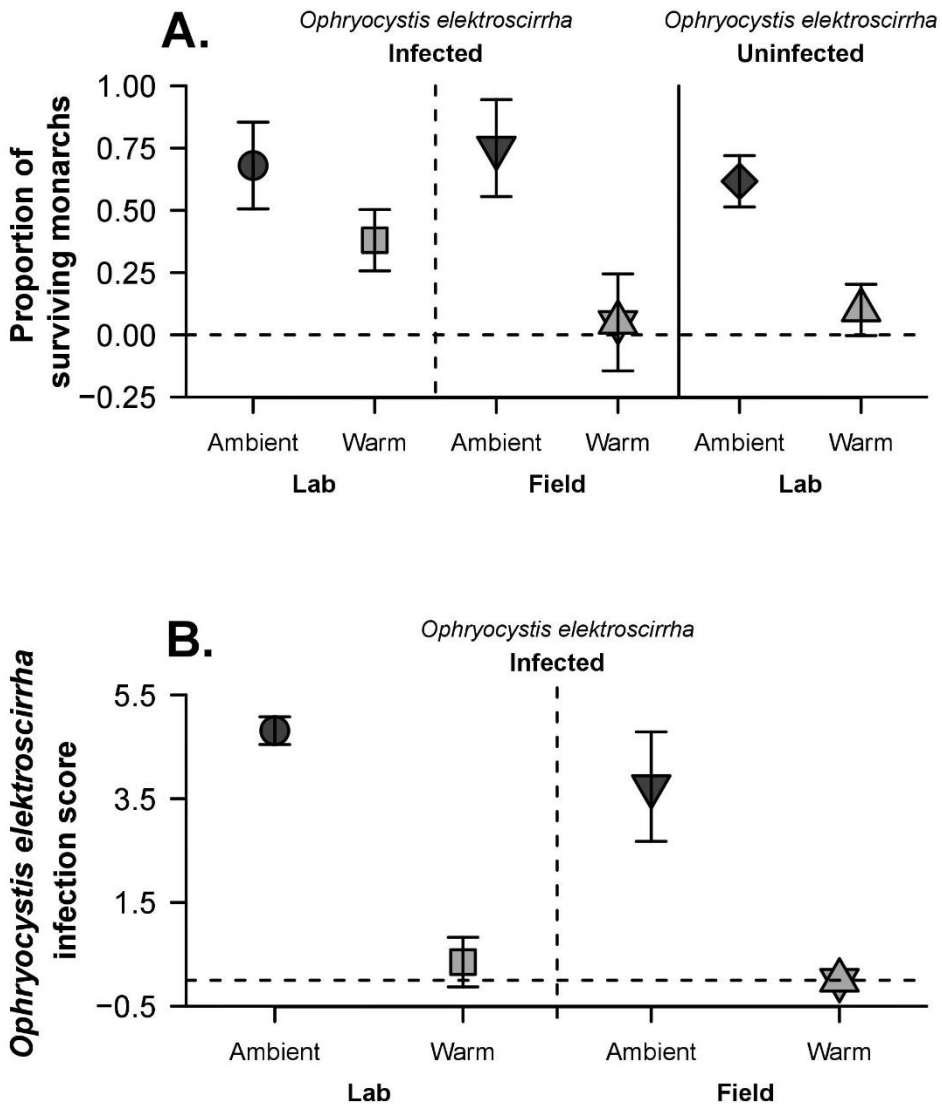

Figure 3.4. The survival and infection status of *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Dark colors represent ambient conditions; light colors represent warm conditions. (A.) The average survival of both monarch butterflies, with 95% confidence intervals. (B.) The average *Ophryocystis elektroscirrha* score for monarch butterflies in the OE-infected experimental runs. Scores were assessed post-experimentation.

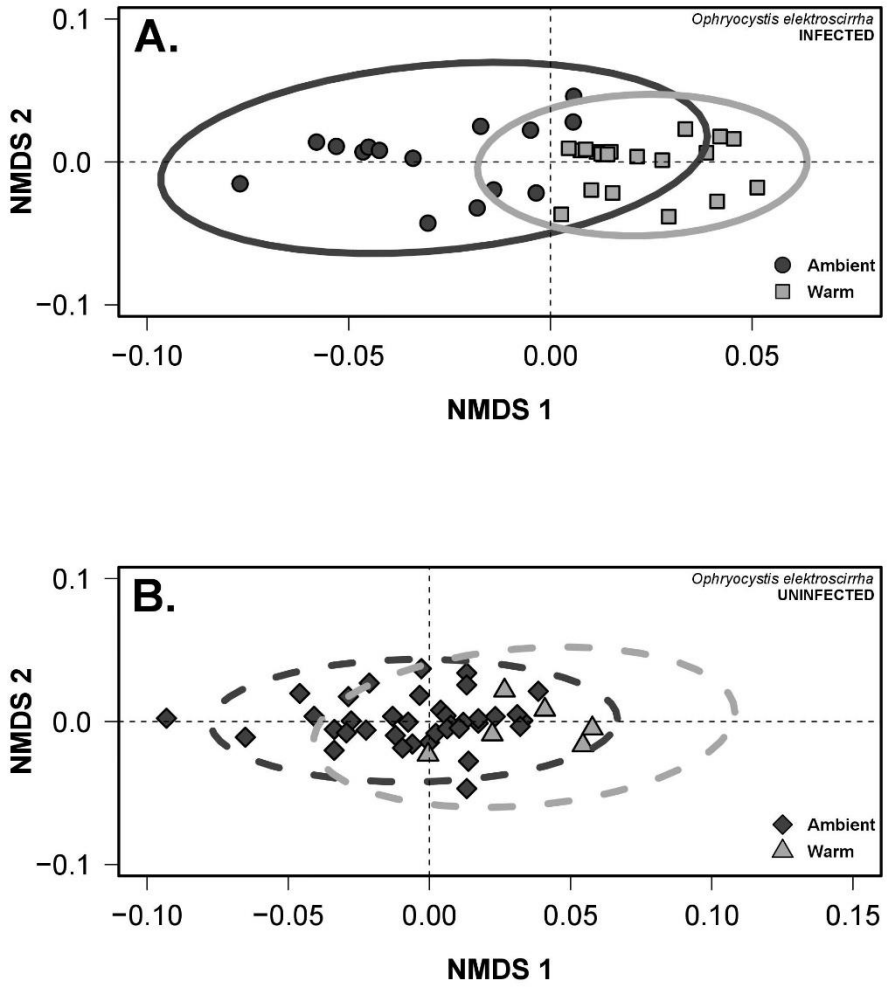

Figure 3.5. Differences between *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Shapes represent individual monarch butterflies placed in ordination space, combining fitness metrics including development times, weights, pigmentation, and size across monarch life stages (e.g., egg, larva, pupa, and adult stages). NMDS axis 1 and NMDS axis 2 aid in visualizing differences across treatments. Ellipses represent 95% confidence interval areas around a centroid point relative to the ordination clustering. Dark colors represent ambient conditions; light colors represent warm conditions. (A.) Shapes represent individual, OE-infected monarch butterflies between warmed and ambient lab conditions. (B.) Shapes represent individual, OE-uninfected monarch butterflies between warmed and ambient lab conditions.

$F_{1,46}$=58.35, p=0.001, Fig. B.3), and marginally interact with experimental site location (PerMANOVA, $F_{1,46}$=4.45, p=0.037, Fig. B.3). When considering different locations, all metrics except true fitness metrics did not differ between sexes. Temperature treatment conditions dramatically impacted overall days-to-development for OE-infected monarchs. Overall, OE-infected monarchs reared in warmer conditions developed 26% slower than those in ambient conditions (temperature conditions, χ_1^2=23.7858, p=0.0017, Fig. 3.6A). Furthermore, pupae (under warm conditions) developed 2% faster than ambient monarchs (temperature conditions, χ_1^2=24.1988, p=0.0017, Fig. 3.6A). Temperature conditions affected monarch weight, namely in that pupae in warmed conditions weighed 20% less than ambient pupae (treatment condition, $F_{1,32}$=51.95, p=0.0017, Fig. 3.6B). OE-infected adult monarchs, in warmed conditions, weighed 27% less than those in ambient conditions (treatment condition, $F_{1,30}$=52.99, p=0.0017, Fig. 3.6B). Furthermore, OE-infected monarch larvae reared under warmed, laboratory conditions were 17% lighter in overall coloration compared to larval monarchs reared under ambient treatment (treatment condition, $F_{1,33}$=82.21, p=0.0017 Fig. B.7A). Furthermore, OE-infected monarch larvae reared in the lab under warm treatment conditions had body coloration that had an 18% greater non-white area compared to monarchs reared under ambient conditions (treatment condition, $F_{1,33}$=264.61, p=0.0017, Fig. B.7B). OE-infected monarch pupae reared under warm conditions had a 1% greater non-white area than pupae reared under ambient temperature conditions (treatment condition, $F_{1,32}$=24.32, p=0.0017, Fig. B.7B). While temperature did not affect overall larvae and pupal lengths (Fig. B.7C), OE-infected

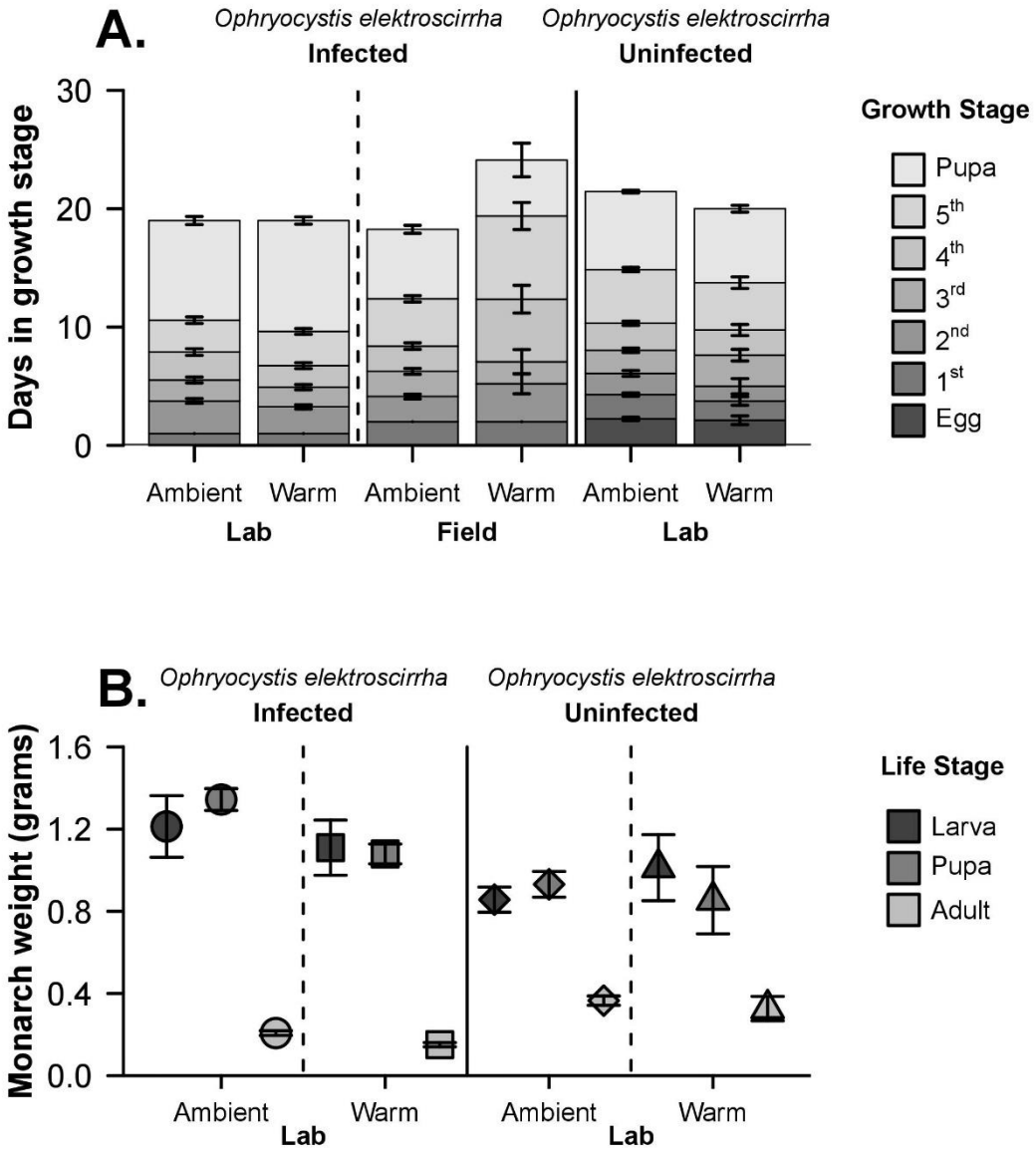

Figure 3.6. The average development time for a specific growth stage (i.e., days-to-development) and average growth-stage masses of *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Colors represent different life stages, and shapes represent different treatment conditions and infection status. (A.) The average days spent in a specific growth stage for monarch butterfly populations, with 95% confidence intervals. (B.) The average growth-stage weights (across larvae, pupae, and adults) for monarch butterflies, with 95% confidence intervals.

pupae reared in warm conditions had an 8% smaller dorsal area compared to pupae reared under ambient conditions (treatment condition, $F_{1,32}$=10.43, p=0.029, Fig. B.7D).

Temperature conditions did interact with experimental locations, affecting metrics of monarch fitness. Across overall days-to-development, lab-reared, OE-infected monarchs under warm conditions developed 9% faster compared to lab-reared monarchs under ambient conditions, while field monarchs at warmed temperatures took 57% longer to develop compared to monarchs reared under ambient conditions (experiment and treatment condition interaction, $F_{1,44}$=87.8, p=0.0006, Fig. 3.6A). 2nd instar, OE-infected monarchs reared under warm conditions in the lab developed 18% faster, while field monarchs at warmed temperatures took 51% longer to develop as 2nd instar (experiment and treatment condition interaction, $F_{1,44}$=11.16, p=0.0051, Fig. 3.6A). Furthermore, 4th instar, lab-reared monarch larvae (in warm conditions) developed 23% faster, while field monarchs at warmed temperatures developed 1.5-times slower than field monarchs in ambient conditions as 4th instar larvae (experiment and treatment condition interaction, $F_{1,44}$=32.79, p=0.0006, Fig. 3.6A). 5th instar, larval monarchs reared in the lab (under increased temperatures) took 6% longer to develop, while field 5th instar monarchs at warm temperatures took 76% longer to develop (experiment and treatment condition interaction, $F_{1,44}$=20.23, p=0.0006, Fig. 3.6A). Finally, OE-infected, lab-reared pupae (under warm conditions) took 10% longer to develop, while monarch pupae in the field (under warm temperature conditions) developed 19% quicker than pupae under ambient field conditions (experiment and treatment condition interaction, $F_{1,44}$=7.1, p=0.0214, Fig. 3.6A).

Experimental location did have a marginal impact on OE-infected monarch fitness metrics. Across overall days-to-development, OE-infected monarchs in the field took 57% longer to develop than monarchs reared in the lab (experiment, χ_1^2=32.4742, p=0.0006, Fig. 3.6A). Specifically, field-reared 3rd instar larvae took 15% longer to develop (experiment, χ_1^2=7.9921, p=0.0141, Fig. 3.6A) and 5th instar larvae took 97% longer to develop compared to lab reared monarchs (experiment, χ_1^2=28.3013, p=0.0006, Fig. 3.6A). Conversely, OE-infected, field-reared pupae developed 40% faster than lab-reared pupae (experiment, χ_1^2=34.5629, p=0.0006, Fig. 3.6A). Overall, while sex did not dramatically drive differences across monarch fitness metrics, during the pupal stage, male pupae weighed 10% more than female pupae (sex, $F_{1,32}$=11.37, p=0.034, Fig. 3.6B).

OE-Uninfected Monarch Fitness Metrics

For OE-uninfected, laboratory monarchs, temperature treatment conditions (either ambient or warmed) did not dramatically impact fitness metrics (PerMANOVA, $F_{1,39}$=1.704, p=0.173, Fig. 3.5B), with monarch sex having no major impact on fitness metrics overall (PerMANOVA, $F_{1,39}$=1.144, p=0.322, Fig. 3.5B). There was a weak effect of temperature condition decreasing adult forewing area, where OE-uninfected adult forewings were 20% smaller when reared under warmed conditions than compared to adult forewings from monarchs reared under ambient environmental conditions (treatment conditions, $F_{1,39}$=11.18, p=0.0486, Fig. B.8D). Additionally, OE-uninfected, male monarchs (in the pupal stage), took 9% longer to develop compared to OE-uninfected females (sex, χ_1^2=21.9745, p=0.0027, Fig.3.6A). Beyond this, there were no differences

across life stages for monarch weights (Fig. 3.6B), pigmentation (Fig. B.8A and Fig. B.8B), length (Fig. B.8C), and most area measurements (Fig. B.8D).

DISCUSSION

Changing environmental conditions, through increased temperatures, can affect all species and their biotic interactions (Karl *et al.* 2009). For monarch butterflies, increased temperatures increase mortality (Zalucki 1982) and developmental times (Lemoine *et al.* 2015), and decrease adult weight (York and Oberhauser 2002). Furthermore, increasing prevalence of *Ophryocystis elektroscirrha* infection in monarchs is causing declines in populations (Belsky and Joshi 2018), and interacts with abiotic and biotic factors, such as with atmospheric CO_2, to decrease milkweed medicinal properties (Decker *et al.* 2018) and with non-native milkweed species to reduce migratory behaviors (Satterfield *et al.* 2015). From our work, we see that warmer environmental conditions and simultaneous OE-infection decrease monarch population growth rates. Throughout a monarch life-cycle, the life-history stage with the most influence on monarch population dynamics is being able to eclose in an adult butterfly from a pupa, and then surviving as a pupa. Furthermore, warmer environmental conditions with concomitant OE-infection across monarch life stages decreased survivorship, developmental times, weights, dorsal area, and increased melanism. Monarchs that were uninfected with OE experienced only marginal impacts on fitness metrics. Yet, even if monarchs are heavily OE-infected (Fig. B.1C), those that do survive warmer temperatures appear to clear the parasites from their systems (Fig. B.1D). This work adds to this body of knowledge by providing empirical support for how climate change and simultaneous parasite infection alter monarch

butterfly population dynamics, while highlighting the need for additional empirical work to fully understand the impacts of climate change and biotic drivers on ecological interactions.

We found that simultaneous parasite infection and increased temperatures decreased monarch population growth and negatively impact aspects of monarch fitness. Regardless of OE-infection status, increased temperatures substantially decreased projected population growth of monarch butterflies (Table 3.3). Monarch butterfly populations have experienced large declines in population size from multiple threats, including climate change (Belsky and Joshi 2018). Here, we were able to empirically quantify the reduction in population growth that increased temperatures have on monarch populations. Furthermore, throughout a monarch life-cycle, the life-history stage that has the greatest impact on monarch population growth is successfully eclosing as an adult butterfly from a pupa, G_{87}, and when factoring in projected fecundity, the most elastic life-history element is pupal survivorship, S_{77}. The population that was an exception to this was OE-infected monarchs reared under warmed conditions in the field, where the most important matrix element to monarch population dynamics was 5th instar larvae successfully pupating, but this is likely driven by the fact that only a single monarch butterfly survived past being a 5th instar larva in this treatment. This result provides an important glimpse into the biology of monarchs by both highlighting the vulnerability monarchs experience while surviving as a pupae and eclosing as an adult, and reinforce that conservation efforts should consider pupal survivorship as an important life stage in assessing ways to manage monarch populations.

Warmer environmental conditions with concomitant OE-infection not only negatively impact monarch population growth, but also lead to decreased survivorship, developmental times, weights, dorsal area, and increase melanism in monarchs. Across experimental locations, monarch survivorship dropped dramatically under warmer environmental conditions, exacerbated by additional OE-infection (Fig. 3.4A). Furthermore, while temperature induced changes in milkweed chemistry may benefit monarchs medicinally by decreasing parasite loads (Lefèvre *et al.* 2010), it seems unlikely that the dramatic declines in monarch survival shown in Fig. 3.4A could be compensated for accordingly. Lab reared, OE-infected monarch butterflies reared under warmed conditions developed significantly faster than monarchs reared under ambient conditions at some instar stages, and slower in others (Fig. 3.6A). Specifically, 2^{nd} and 4^{th} instar larvae developed quicker, while 5^{th} instar larvae and pupae developed slower under warmed conditions. Thus, it appears that while total days-to-development were fewer, overall, for monarchs reared under warmed conditions, the reality is that monarch development is more nuanced than expected, having differential impacts at different life stages. For Lepidoptera, early life stages are most prone to predation, pathogens, and parasitoids (Zalucki *et al.* 2002, Despland 2018). Thus, persistence in these stages could lead to increased mortality, hurting efforts to better manage and increase monarch populations. Additionally, under warmer conditions and OE-infection, monarchs experienced decreased weight and decreased overall area, potentially impacting future adult fecundity, as size is correlated with fecundity in insects (Honěk 1993). Finally, OE-infested, larval monarchs reared under warmer temperatures were overall lighter in color

and had more non-white area than monarchs reared under ambient conditions (Fig. B.7A). While pupae also displayed statistically significant differences, these values are likely biologically irrelevant. With the changes in larval melanism, melanism affects thermal processing in insects (Davis *et al.* 2005), and affects adult mating success (Davis *et al.* 2007). Here, changes in melanism due to increased temperatures may have cascading impacts across larval development and have later consequences for affecting mating success. These results, as a whole, indicate that OE-infection and increased temperatures impart negative effects on monarch butterflies, and despite monarchs displaying a degree of phenotypic responses, these responses may not be enough to overcome the negative, one-two punch of parasite infection and increased environmental temperatures.

One major limitation to our study is that we did not a priori assign an OE spore dose as OE-infection occurred from using wild-caught monarchs. It has been documented that *Ophryocystis elektroscirrha* spores can be successfully passed vertically from infected males or females and horizontally via larval exposure to OE-infected adults (Altizer *et al.* 2004). Additionally, since a dose of 1×10^2 OE-spores is sufficient to illicit OE-infection that negatively impacts monarchs, and given that surviving monarchs reared under ambient conditions were heavily OE-infected (and the OE-infection scale is conservative for heavily infected individuals), all monarchs used were likely bound to become infected with OE upon hatching (Altizer and Oberhauser 1999). Yet, for future work, assigning specified doses of OE spores and tracking the dose-dependent effects with simultaneous climate change on monarch populations would clarify how differing doses of OE impact

dynamics. Furthermore, using OE-spores that differ in virulence across differing strains of milkweed toxicity, within the context of climate change, would also be fruitful to elucidate climate change impacts on the monarch-milkweed-parasite system in a broader context. Furthermore, this study used OE-infected monarchs and OE-uninfected monarchs from different genetic lineages, confounding monarch genetics and OE-infection. Using monarchs all from the same genetic lineage would address this. Additionally, other drivers may also influence these interactions across the monarch-milkweed-parasite system, including water availability (Andrews and Hunter 2015), nutrient deposition (Zehnder and Hunter 2008, Tao *et al.* 2014), elevated atmospheric concentrations of CO_2 (Vannette and Hunter 2014, Decker *et al.* 2018), biotic interactions with milkweed (Satterfield *et al.* 2015), and additional pathogens other than OE (Nifosi and Hunter 2015). Thus, assessing the full impacts of climate change with a suite of changing abiotic and biotic factors proves difficult. Nonetheless, it is clear that concomitant OE-infection with warmed temperatures negatively affect monarch population growth and decreases aspects of monarch fitness.

Finally, hosts and parasites possess unique thermal-performance curves which can show separation from each-other, yet often overlap across temperatures often favoring one organism's performance at the cost of decreased performance to the other (Thomas and Blanford 2003). For example, in a locust-fungal system, locusts that are able to raise their temperatures by fevering can reduce pathogen reproduction by 35%, accrue little to no other negative costs while fevering, and produce viable offspring (Elliot *et al.* 2002). Thus, higher temperatures may favor hosts by both optimizing defense responses and

directly limiting pathogen growth, so long as the temperature extreme does not exceed the thermal tolerance of the host (Thomas and Blanford 2003). Keeping in mind that in addition to increases in average annual temperature, climate models predict increased climatic variability, such as increased frequency of heat waves (Karl *et al.* 2009), higher annual temperatures (and more frequent heat waves) may reduce monarch survival (as temperatures exceed monarch thermal performance limits). Here, monarch butterflies that survived ambient temperature conditions were heavily infected with OE, (Fig. B.1C), and while increased temperatures reduced monarch survivorship, monarchs that did survive the increased temperature conditions were mostly free of OE-infection (Fig. B.1D). Considering that parasite abundance is expected to increase with climate change (Møller *et al.* 2013), we found that increased temperatures from climate change and co-occurring parasite infection will negatively affect host species population growth and fitness. Thus, if parasite abundance is expected to increase with climate change, then potentially the impact of increased temperatures on the long-term virulence of parasites should be assessed to better understand parasite co-extinction risk and climate sensitivity (Cizauskas *et al.* 2017).

Previous studies and methodologies have fallen short on assessing the full impact of climate change on species by neglecting biotic interactions, species evolutionary responses, and direct empirical testing (Pearson and Dawson 2003, Chaianunporn and Hovestadt 2015, Cizauskas *et al.* 2017). Furthermore, recent studies have stressed the importance of assessing the impacts of interspecific interactions (specifically, parasites and hosts) in the context of providing a more clear picture on how climate change will

impact ecological communities (Chaianunporn and Hovestadt 2015, Feldman *et al.* 2017). Through this experiment, we found that parasite infection and increased temperatures together reduce population dynamics and decrease metrics of overall fitness of monarch butterflies, reinforcing that both climate change and parasitism are important drivers in shaping species population dynamics, metrics of overall fitness, and their ecological interactions. Thus, predicted climate change and co-occurring parasite infection may act as a one-two punch to negatively impact species populations; but, it may also be beneficial for some host species as increased temperatures may curb parasite virulence and growth. As the climate continues to change, more empirical evidence is needed to further tease apart the interactions between hosts and parasites which will change in lock-step with a changing climate. These affected biotic interactions and altered abiotic factors will shape ecological communities, and understanding them will improve management practices and help mitigate the potential ecological fallout of climate change.

CHAPTER 4
A CURE FOR INVASIVE SPECIES: IMPLEMENTATION OF A COURSE BASED UNDERGRADUATE RESEARCH EXPERIENCE TO ASSESS COMPETITION OF *ASCLEPIAS CURASSAVICA*, A NON-NATIVE MILKWEED

INTRODUCTION

Science education has rapidly evolved over the last decade, shifting focus from courses that emphasize material memorization towards course curricula that integrate core scientific concepts and competencies with student-centric learning goals often using active learning techniques (AAAS 2011). Hands-on research experience continues beyond the classroom when students enter and work in research labs. These undergraduate research experiences (UREs), especially early in a student's academic journey, boost general scientific literacy amongst undergraduate students (Russell *et al.* 2007). URE participation has been shown to increase student scientific confidence (Seymour *et al.* 2004), conceptual awareness (Hunter *et al.* 2007, Linn *et al.* 2015), engage students from underrepresented groups (Eagan *et al.* 2013), and increase participation in scientific graduate study and careers (Bauer and Bennett 2003). Yet, traditional undergraduate research opportunities are often limited (Russell *et al.* 2007). UREs necessitate close advisory supervision in a research lab, and due to a limited number of university labs on a given campus, are frequently selective and highly competitive (Linn *et al.* 2015). Often, students that do engage in research experiences are graduation-ready upper level students (Linn *et al.* 2015). Considering the limited number of URE positions available at a given time, this may also perpetuate inequities in research communities through a lack of

awareness of research experiences and their benefits, financial and personal barriers, the "rising star" selection process, and societal biases (Bangera and Brownell 2014).

Course-based Undergraduate Research Experiences (CUREs) offer students opportunities to be highly engaged in iterative scientific practices, collaboration, scientific topics, discovery, and research inclusivity (Auchincloss *et al.* 2014, Bangera and Brownell 2014, Brownell *et al.* 2015). These experiences also allow investigators to ask and answer research questions in a manner similar to a citizen science based approach while teaching the next generation of scientists. Thus, CUREs represent a boon to both scientific research and education. Furthermore, CUREs can engage a much greater number of undergraduate students than a traditional URE, with specific emphasis on engaging early division (1st and 2nd year) students in scientific research (Linn *et al.* 2015, Bakshi *et al.* 2016). At Louisiana State University (LSU), CUREs implemented in the 1st year biology laboratories focus on deepening students' understanding of the scientific process while emphasizing reading and understanding primary scientific literature, data analysis and interpretation, and effective communication of scientific data through written reports and poster presentations (Bakshi *et al.* 2016). The ecology CURE discussed here emphasizes the steps of the scientific method throughout an entire semester, while addressing a novel, relevant scientific question (Bakshi *et al.* 2016). While CUREs have been proposed as way to move science instruction forward by positively impacting students' conception about science (Brownell *et al.* 2015), quantifying the impact CURE participation has on students compared to a traditionally structured, lecture based course is lacking and necessary (Auchincloss *et al.* 2014, Laungani *et al.* 2018).

Traditional ecology laboratories at LSU cover invasive species during a couple of weeks over a semester-long course (Figure C.1). Through these weeks, upper-division ($3^{rd}/4^{th}$) students learn that invasive species are exotic species that cause economic harm, environmental harm, or harm to human health (Mack *et al.* 2000, Pimentel *et al.* 2005), and perform small-scale, predesigned, highly-controlled experiments. Invasive species research provides excellent opportunities to study not just invasiveness but also basic biological processes (Sakai *et al.* 2001). This can be further enhanced by spotlighting the impact invasive species have on charismatic species (i.e., species that have broad public appeal) (Albert *et al.* 2018).

In the United States, Monarch butterflies (*Danaus plexippus*) are a highly charismatic insect species well known because of their use in K-12 education (Matthews *et al.* 1997, Eick 2012), citizen science engagement (Howard *et al.* 2010), pollinator status (Brower *et al.* 2006), and for their annual, 3500-km, multi-generational migration (Brower and Malcolm 1991). Furthermore, monarch butterflies are a specialist species, meaning their host plants on which their larvae develop belong to a single genus *Asclepias*, the milkweed. Thus, monarchs are sensitive to changes in the community composition of their milkweed resource (Ali and Agrawal 2012). Monarch populations have experienced historic population declines due to multiple interacting factors, one major factor being milkweed habitat loss (Belsky and Joshi 2018). Milkweed pollinator gardens may help increase population numbers by improving oviposition rates (Cutting and Tallamy 2015, Belsky and Joshi 2018). But, an invasive milkweed species being preferentially sold and planted may negatively impact monarchs by reducing their need for migration, increasing

parasite prevalence in non-migratory populations, and forming ecological traps (Satterfield *et al.* 2015, Faldyn *et al.* 2018). While milkweed gardens improve monarch larva performance compared to natural sites (Cutting and Tallamy 2015), the species of host milkweed planted and plant arrangement need to be considered. Given the prevalence of *A. curassavica* being sold in stores, understanding how this invasive milkweed, *A. curassavica*, may compete with native milkweed species, such as *A. incarnata* and *A. tuberosa*, when planted together in milkweed gardens is of crucial importance. If *A. curassavica* continues to be preferentially planted across large spatial scales and outcompete native species, migratory monarch butterfly populations may be adversely impacted (Satterfield *et al.* 2015, Faldyn *et al.* 2018, Decker *et al.* 2019).

While important from a research perspective, ecological "hot" topics (e.g., invasive species) provide researchers with a unique opportunity to both engage undergraduate students in authentic research while also addressing important ecological questions. From the student's perspective, having the opportunity to be engaged with real scientific research studying the interconnectedness between a charismatic species (i.e., monarch butterfly) and an ecological "hot" topic (i.e., invasive species ecology) should increase student perception and engagement with the course material more than a traditional, lecture based course. Through the design and implementation of a CURE at LSU, we investigated how CURE participation leads to improved understanding, awareness, and perception of an ecologically relevant topic in early-division undergraduate students compared to a traditionally structured ecology laboratory comprised of upper-division students. Additionally, to better understand the ecology of invasive species and how this

relates to best management practices, we asked: what are the competitive effects of the invasive milkweed, *A. curassavica*, on two Southeastern United States, native milkweed species, *A. incarnata* and *A. tuberosa*? Combined, this study will help provide clarity on the impact of conducting authentic research in CUREs while elucidating the competitive interactions of an invasive species to improve applied management practices.

MATERIAL AND METHODS

MILKWEED

Monarch butterflies are a specialist species that almost exclusively oviposit on milkweed species within the genus *Asclepias* and have a wide distributional range across North America (Urquhart and Urquhart 1978). By feeding on *Asclepias*, monarchs are able to sequester toxic cardenolides, which the plant produces as a chemical defense, and use these cardenolides for themselves as anti-predator and anti-parasite defenses *(*Brower *et al.* 1967, de Roode *et al.* 2008). Besides differing in the amount of cardenolides produced, *Asclepias* species differ in latex exudation (Agrawal and Konno 2009), leaf morphology (Agrawal *et al.* 2009a), and general phenologies (Woodson 1954). *A. incarnata* is a common, herbaceous, perennial milkweed species native throughout the eastern and southeastern portion of the monarch migratory range that senesces during the winter months and possesses a less complex level of morphological modifications compared to other milkweed species (Woodson 1954, Ladner and Altizer 2005). *A. tuberosa*, another native species, is found extensively throughout the mid-west and southeastern portions of the United States (Woodson 1954). *A. tuberosa* is a herbaceous, perennial milkweed that forms a deep, thick, and woody rhizobial rootstalk with crowded, irregular leaves

(Woodson 1954). In contrast, *A. curassavica* is an exotic, preferentially planted and commercially-produced, herbaceous, perennial milkweed species found predominantly in the southeastern United States and is native to South America and northern Mexico. *A. curassavica* produces 36-times the amount of toxic cardenolides the native milkweed species produce (Malcolm and Brower 1986), can negatively affect monarch butterflies by providing a year-round source of food that reduces the propensity to migrate (thereby increasing disease prevalence in non-migratory populations) (Satterfield *et al.* 2015), and can act as an ecological trap for monarch butterflies in the context of climate change (Faldyn *et al.* 2018). *A. incarnata*, *A. tuberosa*, and *A. curassavica* are commonly planted together in monarch gardens and may compete in natural settings (Baker and Potter 2018).

CURE AND ECOLOY LABORATORY STUDENTS

While survey data were collected from the Spring 2018 CURE only, the CURE was offered for three total semesters (Fall 2017, Spring 2018, and Fall 2018) with two sections of early-division biology laboratories offered per semester at LSU. Each section had thirty-two enrolled students (forty-eight enrolled survey respondents, Table 4.1), enrolled students did not self-select into the course (i.e., students did not choose to enroll in the CURE course over a regular lab), and enrolled undergraduate students received training in basic biological and research skills that they will utilize both directly and indirectly in other courses or post-graduate studies. This CURE was developed using the framework described in Bakshi et al. (2016), and had been successfully applied to a microbiological

Table 4.1. Respondent demographic information for early-division CURE laboratory, upper-division Ecology laboratory, and the upper-division Ecology lecture course.

		Early-division CURE	**Upper-division Ecology Laboratory**	**Upper-division Ecology Lecture**
	Total no. of students	48	---	130
	Respondents	32 (67%)	13 (39% of Lecture respondents)	33 (25% of total)
Class standing	1st year	10 (31%)	0 (0%)	0 (0%)
	2nd year	2 (6%)	0 (0%)	0 (0%)
	3rd year	0 (0%)	0 (0%)	7 (21%)
	4th year	0 (0%)	13 (100%)	26 (79%)
Ethnicity	Other	4 (13%)	9 (69%)	7 (21%)
	White	28 (88%)	4 (31%)	26 (79%)

curricula (Bakshi *et al.* 2018). Using this framework, we designed the CURE for a semester structure, with CURE sections meeting for three hours, once a week, for thirteen weeks.

The entire course work-flow does not necessitate thirteen weeks, and can be modified. The project focused on assessing the competitive interactions between an invasive milkweed species, *A. curassavica*, and two Louisiana, native milkweed species, *A. incarnata* and *A. tuberosa*. Objectives for this CURE are to:

1. Describe the relationship between research objectives and experimental design through the scientific method;
2. Learn macrobiological content (e.g., studying large, living organisms), relevant ecological theory, and Louisiana natural history;
3. Locate, read, and use scientific literature in conjunction with using and interpreting statistics; and,
4. Improve communication skills to all audiences through report writing, social media assignments, and scientific posters.

The CURE curricula can be subdivided into six, separate sections highlighting each step of the scientific method (Figure 4.2 and Figure 4.3):

1. **Pattern Identification**: At the start of the semester, CURE curricula included basic invasive species biology, monarch butterfly life-history, and milkweed biology, allowing students to identify patterns that exist between the invasive *A. curassavica* and native milkweed species (Figure 4.3, Week 1).
2. **Hypotheses proposal**: Prior biological concepts, with reading and discussing scientific literature on invasion biology principles, helped students form

hypotheses on the competitive interactions between *A. curassavica* and the native milkweed species (Figure 4.3, Week 2).

3. **Create predictions**: Through physical manipulation of the study species, wherein students handled and planted the milkweed plugs used in the competition experiment, CURE students developed testable hypotheses (Figure 4.3, Week 3).
4. **Hypothesis testing**: Students tested their hypotheses by collecting initial milkweed fitness metrics, learned and applied appropriate statistics, then collected final milkweed fitness metrics after allowing the milkweed plants to grow for six weeks (Figure 4.3, Week 4-8).
5. **Interpreting results**: Small groups interpreted their results by analyzing the final milkweed fitness metrics, and began communicating those results through formal scientific writing and scientific posters (Figure 4.3, Week 9).
6. **Communicating results**: Student-led, small groups communicated their science to the class, then to other CURE students and the public at the end-of-semester CURE Symposium (Figure 4.3, Week 10-13).

Throughout the semester, CURE students were actively involved in learning the material and communicating the results. While the curriculum was structured iteratively to allow for students to improve their research principles, this CURE was also designed as a communication-intensive course and designated as such by LSU's Communication Across the Curriculum (a multifaceted program that works to improve the writing, speaking, visual and technological communication skills of LSU undergraduates), emphasizing a course-wide communication initiative (Figure 4.2). The course

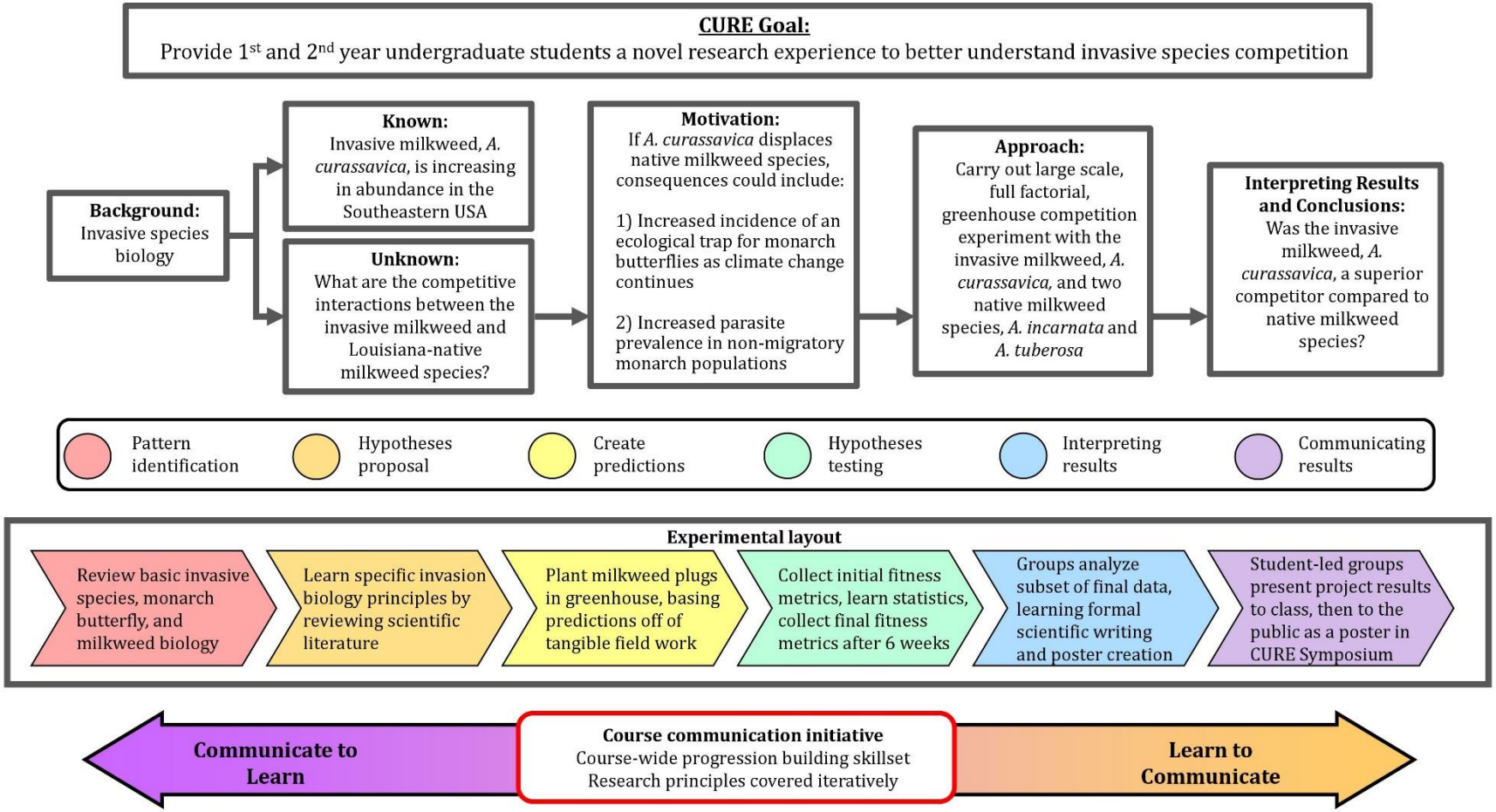

Figure 4.2. Flowchart of the CURE course layout and experimental design. Throughout the course, students are guided through the steps of the scientific method to gain understanding, relevance, and importance of the project and science broadly. Colors (red, orange, yellow, green blue, violet) correspond to the steps of the scientific method. Specific assignments are outlined in Fig. 4.3. This flowchart can be modified to suit projects using a similar course design.

Week	In-Class Objectives	Activites	Assignments
1	Background and introduction to research questions; experimental design	Tour greenhouse and review experimental layout	Assign ICWA1 (in-class writing assignment 1; read Jeschke et al 2014) - monarch documentary review
2	Reading scientific literature; read Jeschke in class; discuss Jeschke in groups then class; social media and science	Main points in scientific papers; other research papers introduced	Assign take-home assignment (THA) 1 (leading questions for Jeschke 2014); social media assignment (SMA) 1 (Instagram collage due after last data collection); ICWA2 due
3	Plant evolution; species interactions	Experimental design/research paper discussion; plant milkweed in greenhouse	Quiz 1; THA1 due
4	Data collection I (initial); experimental design review; planting and scientific record keeping	Experimental design discussion (paper methodology); tour local lake area to identify invasive species and impacts	Quiz 2; assign papers to groups for lightning talks; ICWA3 - LSU lakes tour worksheet
5	Lightning talks and live tweeting; visit LSU Natural History Museum to view specimens and natural history	Lightning talks	Quiz 3; SMA2 (live tweeting and lightning talk); assign THA2 (intro and methods; due next class)
6	Scientific writing; vist LSU Herbarium to see collections and identify local invasive species	Evaluate scientific papers	THA2 due; assign THA3
7	Data analysis I - frequentist statistics assignment		THA3 due; assign formal writing assignment (FWA) 1 (intro and methods)
8	Data collection II (final)		Quiz 4; FWA1 due; ICWA 4 due (badger data)
9	Above and below biomass; data analysis II (milkweed data); poster design, development, and critique	Critique posters	Quiz 5; ICWA 5 (own data) assigned; THA5 (poster critique) assigned
10	Tour of local Bluebonnet Swamp nature center; identify invasive species, impacts, and wildlife in natural setting	Have fun viewing and learning about Louisiana natural history!	Quiz 6; ICWA 5 due (own data); THA4 assigned (reflective writing about invasives/swamp); FWA2 assigned (results and discussion)
11	Final exam review; poster presentations; peer evaluation and reflection		FWA2 (results and discussion - 1 per group) due; THA4 due; THA5 due (poster critique)
12	Final exam	Final exam; final versions of poster due	
13	CURE symposium		SMA1 due (Instagram post)

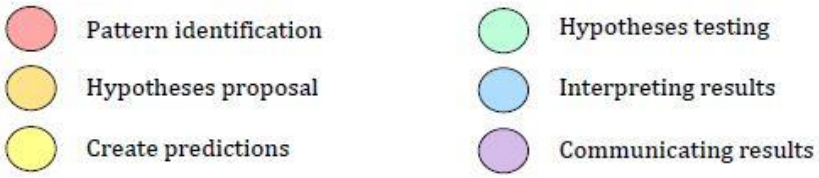

Figure 4.3. Class syllabus for CURE during the Spring 2018 semester. Course structure based on framework outlined by Bakshi *et al.* (2016). Colors correspond to the steps in the scientific method covered throughout the project and throughout the semester.

assignments were structured in a way where students communicated material to learn specifics about invasive species and the project as a whole at the beginning of the course.

By the end of the course, the focus turned to students learning how to communicate the results of the project to broader audiences (Figure 4.2). For example, early-semester projects such as discussing primary literature, lightning talks (i.e., focused presentations on a selected journal article), in-class discussions, and writing assignments reinforced student learning through communication (Figure 4.3, Appendix D). End-of-semester assignments, such as presentations of results, in-class poster presentations, and the final CURE symposium presentations, improved student communication skills and built on previously learned material (Figure 4.3, Appendix D).

Ecology laboratory at LSU was offered in seven different sections, and follows a curriculum that covers major ecological topics presented in lecture (Figure C.1). The ecology lab covers invasive species biology during weeks #9- #12, wherein students designed small scale experiments guided by the instructor using the invasive swamp plant, elephant ear (*Colocasia esculenta*). The experiments designed are not necessarily designed to address novel scientific questions, rather, they are designed to help upper division students learn the basic ideas of hypothesis testing, data collection, data analysis, and report writing.

EXPERIMENTAL SETUP

Milkweed Competition Experimental Design

Students conducted a fully factorial greenhouse experiment to examine competition between the invasive milkweed, *A. curassavica*, and two Louisiana, native

milkweed species, *A. incarnata* and *A. tuberosa* (Table C.1, Figure C.3). *A. curassavica* plugs were ordered from Cleggs Nursery (Baton Rouge, LA, USA), and *A. incarnata* and *A. tuberosa* plugs were ordered from North Creek Nursery (Landenberg, PA, USA). *A. curassavica* plants were trimmed prior to planting to match the aboveground and belowground biomass of the *A. incarnata* and *A. tuberosa* plugs, and *A. incarnata* and *A. tuberosa* plugs were brought out of dormancy or allowed to acclimate in environmental growth chambers (Conviron CMP6010) set at 16-hr photo-periods at 28°C. There were seven competition treatments in total: *A. curassavica* grown alone, *A. incarnata* grown alone, *A. tuberosa* grown alone, *A. curassavica* and *A. incarnata* grown together, *A. curassavica* and *A. tuberosa* grown together, *A. incarnata* and *A. tuberosa* grown together, and all three species grown together, with each treatment being replicated seven times (Figure C.10). There were a total of forty-nine, approximately 40-quart plastic containers, with six plants in each container in a 3 x 2 array regardless of treatment (allowing for consistent plant density in each container) (Figure C.10). The experiment was performed in an enclosed LSU AgCenter Horticulture greenhouse (Louisiana State University, Baton Rouge, LA, USA), with the containers spaced evenly throughout (Figure C.3A, Figure C.11). The greenhouse was equipped with a drip sprinkler system, and each container was watered daily. Students planted the milkweed plants in topsoil with Scotts Osmocote 14-14-14 fertilizer. Initial data collection was considered a practice run to measure basic plant fitness metrics. Milkweed data were collected across the Fall 2017, Spring 2018, and Fall 2018 semesters after six weeks of growing together. While surveying students occurred only during the Spring 2018 semester, results from milkweed data collected during the

Spring 2018 semester were the same as the results from milkweed data pooled across all three semesters (Figure C.4). To maximize sample size, all three semesters of milkweed data were analyzed together. Data collection was designed to be easily reproducible, affordable, and comprehensive, with nine plant metrics collected for each individual milkweed (number of stems, number of leaves, flower count, plant height, stem length, latex exudation, leaf toughness, aboveground biomass, and belowground biomass). The number of stems, number of leaves, and flower counts were counted using tally counters and overall plant height with individual stem length measured using metric rulers. Milkweed latex measurements were adapted from Agrawal (2005), wherein a fully expanded, intact leaf was removed and the exuding latex was collected on a dried, preweighed disk of filter paper, then placed and sealed inside a dried, preweighed Eppendorf vial. The vial was weighed in the lab, and the resulting difference was recorded as the amount of latex exuded. Leaf toughness was measured using rip-o-meters; a cheap, easily reproducible method for assessing leaf toughness (Campbell *et al.* 2004). Here, a small, plastic mouthwash cup (with one hole punched near the lid and an unwound paper clip inserted through the hole) was used to puncture the middle most point of a leaf directly under the midrib, filled with sand, and once it ripped through the leaf, was then weighed (Campbell *et al.* 2004). Requiring two students at a time, one student would hold the leaf taut with the rip-o-meter suspended under the midrib, and a second student slowly filled the cup with sand and caught the rip-o-meter when it ripped through the leaf, recording the final weight. The recorded weight approximates leaf toughness (e.g., a tougher leaf takes more weight to rip). Lastly, to measure biomass, plants were clipped at

the base. The vegetative tissue collected and weighed to quantify wet aboveground biomass, while root tissue was collected, washed, and weighed to measure wet belowground biomass.

Assessing CURE and upper-division Ecology student opinions

Student understanding and perceptions of invasive species were assessed using a pre-post survey approach. The study population consisted of 1st and 2nd year undergraduate students enrolled in the CURE (early-division CURE students) and 3rd and 4th year students enrolled in a traditionally-structured ecology laboratory and traditionally structured ecology lecture course (upper-division Ecology laboratory/lecture). Students were surveyed during the Spring 2018 semester. Both courses had a similar number of respondents to the survey, but a greater proportion of respondents compared to class enrollment were from the CURE course (Table 4.1). The survey instrument, which was approved by the LSU Institutional Review Board, was modified from and approved for use by (Lauber *et al.* 2015). Lauber et al. (2015) surveyed New York state citizens about invasive species and their own perceptions. Changes in the survey were made to reflect questions more relevant to students in Louisiana (Appendix D). The survey quantifies respondents' perceptions on invasive species across five main categories:

1. **Beliefs**: Focused on assessing students' preconceived notions about and knowledge of invasive species biology and invasive species impacts (See question 4 in Appendix S2:1).
2. **Concerns**: Assessed student worries about the relationship between the impacts of invasive species, on societal issues (See question 5 in Appendix S2:1).

3. **Drivers**: Gauged student knowledge about invasive species vectors and the relationship of common activities that influence invasive species spread (See question 12 in Appendix S2:1).
4. **Behaviors**: Focused on assessing student willingness to change their behavior if they found out their activities could lead to the spread of invasive species (See question 13 in Appendix S2:1).
5. **Contributions**: Assessed if students understand their contributions to invasive species spread, to what degree would they be willing to modify those contributions, and what kind of considerations could influence student willingness to change behaviors (See question 14 in Appendix S2:1).

Each category had a series of questions, followed by Likert responses (Appendix S2:1). Survey respondents can answer Likert responses with a degree of specificity that reflects their level of agreement or disagreement, across a symmetric scale, where the intention is to capture respondents' true feelings towards a particular topic (Likert 1932). These potential responses may be listed as: not concerned at all, slightly concerned, moderately concerned, and very concerned, representing a bipolar scale from negative to positive feelings about the question being asked. Initial surveys were opened for one week allowing student responses after the second week of classes for both courses, and final surveys were opened for one week allowing student responses after the CURE final, but before the 3rd and 4th year upperclassmen ecology course final exam. This way, initial survey respondents had minimal exposure to invasive species material, and final survey

respondents had completed either the entirety of the CURE course or the entirety of classroom lectures for the ecology course.

STATISTICAL ANALYSES

Milkweed competition metrics

Milkweed fitness metrics were analyzed using a permutional MANOVA performed in R using 'adonis' in the 'Vegan' package (Oksanen *et al.* 2015). This was performed comparing differences across the independent variables of the three milkweed species, then between each competition treatment alone. To include data across all three semesters, the dependent variables consisted of the number of stems, number of leaves, plant height, average stem length, and the amount of latex exuded (*A. curassavica* included number of flowers as well). The results remain the same if the analysis is expanded to include aboveground biomass, belowground biomass, and leaf toughness, but this limits the data only to the Spring 2018 semester (Figure C.4). To assess differences across milkweed fitness metrics, we first used metaMDS in 'Vegan' for non-metric multidimensional scaling (NMDS) (McCune and Grace 2002) with 999 permutations per model run and a maximum of 60 runs per dimension. Following that, a permutational MANOVA was used and acts as an analysis of variance by partitioning among sources of variation and fitting linear models to calculated distance matrices based on these partitions (Oksanen *et al.* 2015). Model stress declined rapidly from a one-dimensional to a two-dimensional model, declining only slightly thereafter in a three-dimensional model. Model stress is a goodness of fit statistic for the observations, defined so that the sum of squared values is equal to squared stress where large stress values

indicate a poor model fit (e.g., stress value between 0.1-0.2 is a good fit) (Oksanen *et al.* 2015). When comparing between milkweed species, we used a two-dimensional model (model stress = 0.15), indicating a good ordination fit. When comparing the impacts of competition treatment across milkweed species, we used a two-dimensional model for *A. curassavica* competition (model stress = 0.2), *A. incarnata* competition (model stress = 0.13), and for *A. tuberosa* competition (model stress = 0.15), all indicating good ordination fits. We used the NMDS coordinates from these analyses to plot the milkweed fitness metrics in multidimensional space. Mixed-effect ANOVAs were used to analyze milkweed differences across competition treatments, and non-parametric Kruskal-Wallis tests were used to analyze differences when the data did not fit a normal distribution (SAS Institute Inc 2013). The semester of data collection was classified as a random effect. A sequential bonferroni correction was applied to the milkweed comparisons using the proc multtest procedure (Rice 1989, SAS Institute Inc 2013).

Early-division CURE and upper-division Ecology course surveys

Course-survey data were analyzed in SAS 9.4 (SAS Institute Inc 2013). Likert responses were averaged for each response category, as multiple questions framed in the same context better captures student opinions (Likert 1932). Repeated measure, mixed effects ANOVAs were used to analyze responses using the proc mixed procedure with each respondent's course section classified as a random effect (SAS Institute Inc 2013). The proc mixed procedure accounts for both paired survey responses and unpaired responses. All data were tested to ensure normality using the proc freq procedure (SAS Institute Inc 2013). Survey responses from students enrolled in upper-division ecology

laboratory violated normal tests of normality, likely due to a low response rate and sample size. Yet, reviewing the normal quantile plots indicated each comparison approximated normality. Survey responses for the lecture-portion of the ecology course that were extreme outliers, assessed by reviewing the normal quantile plot and residual values, were removed to ensure normality with a larger sample size. For behavior responses, variability and a single-answer response for violated formal tests of normality, despite a logarithm transformation (shapiro-wilk = 0.0064), but reviewing the transformed normal quantile plot indicated an approximation to normality.

RESULTS

MILKWEED COMPETITION METRICS

Milkweed Survivorship

Nearly 100% of the *A. curassavica* plants survived across all treatments, a 15% and 40% increase in plant survivorship compared to the native *A. incarnata* and *A. tuberosa* plants, respectively (χ^2_2=53.3977, p<0.0001, Figure C.5A). Within all competition experiments, individual *A. curassavica* plant survival was unaffected when grown in competition (Figure C.5B). Furthermore, neither individual *A. incarnata* or *A. tuberosa* plant survival was impacted when grown with the other native milkweed species, the invasive *A. curassavica*, or with both species (Figure C.5C and Figure C.5D).

Differences between Asclepias sp.

Across the Fall 2017, Spring 2018, and Fall 2018 semesters, the three species of milkweed displayed striking differences across fitness metrics with *A. curassavica* being the most robust species (PerMANOVA, $F_{5,546}$=35.523, p=0.001, Figure 4.4A). *A. curassavica*

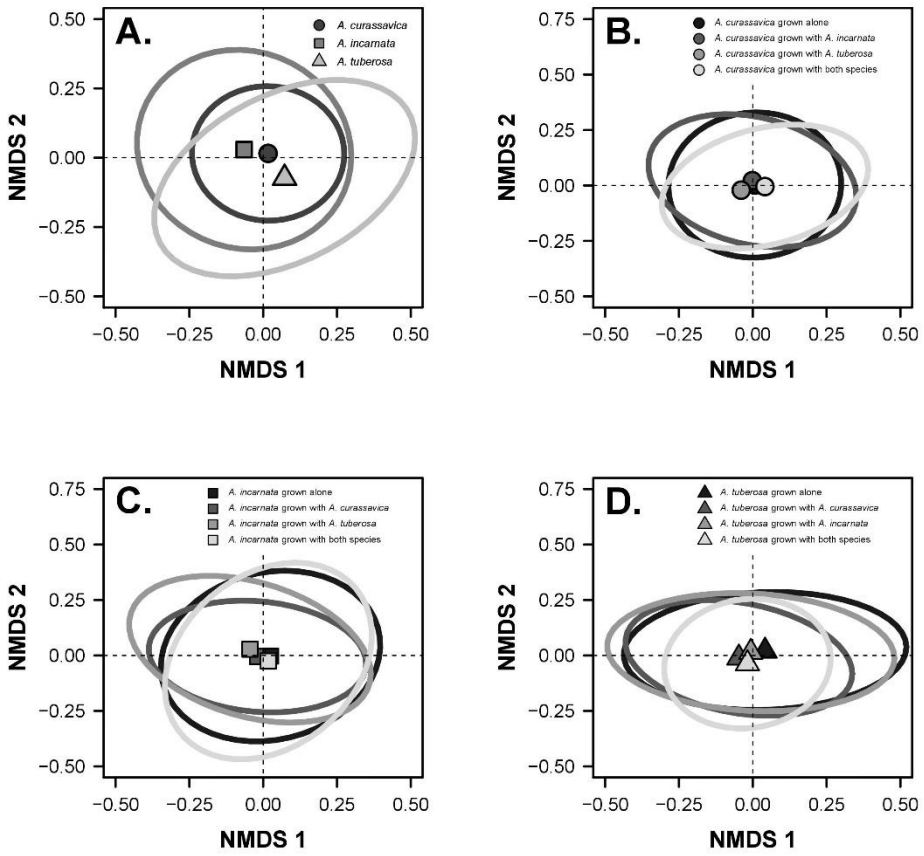

Figure 4.4. Shapes represent individual milkweed placed in ordination space. NMDS axis 1 and NMDS axis 2 aid in visualizing the differences that occur across individual *A. curassavica*, *A. incarnata*, and *A. tuberosa* milkweed plants as separate species across all treatments and when grown in competition. All ordination points represent centroid point for each NMDS cluster. Ellipses represent 95% confidence interval areas around a centroid point. (A.) Shapes represent the different milkweed species. From the separation of each centroid point, milkweed species metrics are very different between the three species. Together, each species is physically distinct from each other, with *A. curassavica* being the most robust species across fitness metrics. (B.) Shapes represent *A. curassavica* plants, while shading represents the competition between *A. curassavica* and native species. From the separation of *A. curassavica* grown with native species, *A. curassavica* in competition produced a greater number of stems, a greater number of flowers, and marginally longer stems. (C.) Shapes represent *A. incarnata* plants, while shading represents competition with *A. incarnata*. From the lack of separation between ordination points, there is little effect of competition on *A. incarnata*. (D.) Shapes represent *A. tuberosa* plants, while shading represents the exposure of *A. tuberosa* to different competition treatments. From the lack of separation between *A. tuberosa* ordination points, there is little effect of competition on *A. tuberosa*.

produced more than twice the number of stems (χ^2_2=187.89, p=0.0009, Figure C.6A) and nearly twice the number of leaves (χ^2_2=226.42, p=0.0009, Figure C.6B) compared to either native species. Furthermore, *A. curassavica* produced 13-times more flowers than either native milkweed species (χ^2_2=191.88, p=0.0009, Figure C.6C). On average, *A. curassavica* plants were more than twice as tall (χ^2_2=366.58, p=0.0009, Figure C.6D) and produced stems that were approximately 65% longer than either native milkweed species (χ^2_2=186.31, p=0.0009, Figure C.6E). Both *A. curassavica* and *A. incarnata* exuded more than twice the amount of latex as *A. tuberosa* (χ^2_2=33.68, p=0.0009, Figure C.6F). *A. curassavica* produced leaves that were 2.5-times tougher than leaves produced by either *A. incarnata* and *A. tuberosa* (χ^2_2=155.71, p=0.0009, Figure C.6G). Finally, *A. curassavica* produced nearly 5-times the amount of aboveground biomass (χ^2_2=139.16, p=0.0009, Figure C.6H) and more than 7.5-times the amount of belowground biomass than either native milkweed species (χ^2_2=114.45, p=0.0009, Figure C.6I).

Competitive interactions between Asclepias sp.

Competitive impacts on milkweed grown in competition were most apparent in *A. curassavica* and minimal for *A. incarnata* and *A. tuberosa*. *A. curassavica* developed striking differences across competition treatments (PerMANOVA, $F_{3,221}$=2.406, p=0.007, Figure 4.4B). *A. curassavica* grown with both native species produced a minimally greater number of stems (χ^2_3=12.6, p=0.0448, Figure C.7A) but nearly 2.5 times more flowers (χ^2_3=11.8, p=0.0574, Figure C.7C). *A. curassavica* plants grown either alone or with both native species produced stems that were 20% longer than *A. curassavica* plants grown with only *A. incarnata* or *A. tuberosa* (χ^2_3=14.6141, p=0.0198, Figure C.7E). There were no

differences in the average number of stems, leaves, flowers, plant height, amount of latex exuded, leaf toughness, aboveground, or belowground biomass in *A. curassavica* plants grown in competition with either native plant species (Figure C.7). Native *A. incarnata* plants grown in competition treatments showed no differences across plant metrics (PerMANOVA, $F_{3,198}$=1.852, p=0.052, Figure 4.4C). *A. incarnata* plants showed no differences in the average number of stems, leaves, flowers, plant height, stem length, amount of latex exuded, leaf toughness, aboveground, or belowground biomass regardless of being grown in competition with the invasive *A. curassavica* or *A. tuberosa* (Figure C.8). Lastly, *A. tuberosa* plants showed no impacts on plant metrics when grown across competition treatments (PerMANOVA, $F_{3,125}$=1.312, p=0.219, Figure 4.4D). There were no meaningful differences in the average number of stems, flowers, plant height, amount of latex exuded, leaf toughness, aboveground, or belowground biomass in *A. tuberosa* plants grown in competition with either the invasive *A. curassavica* and *A. incarnata* milkweed species (Figure C.9).

EARLY-DIVISION CURE AND UPPER-DIVISION ECOLOGY LABORATOY RESPONSES

Overall, early-division undergraduate CURE students experienced concomitant changes in perceptions towards invasive species similar to upper-division ecology laboratory students. There were no differences in initial survey responses between early-division undergraduate CURE students compared to upper-division ecology laboratory students. Participation in either course led to a 7.5% increase in student beliefs about the negative effects of invasive species (trial, $F_{1,29.3}$=6.09, p=0.0197, Figure 4.5A). Similarly, participation in either course increased student concerns about invasive species by 8%

(trial, $F_{1,22.2}$=12.51, p<0.0018, Figure 4.5B). Interestingly, student participation in the early-division undergraduate CURE led to a marginal 12% increase in student understanding of the ecological mechanisms that drive invasive species ecology compared to upper-division ecology laboratory students (course, $F_{1,45.8}$=3.85, p=0.0558, Figure 4.5C). Furthermore, enrollment in both courses led to a 12% in student understanding of the ecological ideals that underpin invasive species (trial, $F_{1,37.3}$=6.6, p=0.0144, Figure 4.5C).

Over the course of the semester there were no differences between early-division undergraduate CURE students and upper-division ecology laboratory students in willingness to change behaviors that contribute to invasive species spread compared to (Figure 4.5D). Finally, enrollment in either course through the semester led to a 6% increase in student understanding of their contributions to invasive species spread and how invasive species impact their personal lives (trial, $F_{1,23.9}$=8.32, p<0.0082, Figure 4.5E).

DISCUSSION

MILKWEED: *ASCLEPIAS CURASSAVICA* AS A COMMENSAL SPECIES

Student-driven, CURE research through a species interaction experiment directly quantified the performance of the adventive milkweed species, *A. curassavica*, and the commensalistic interaction when grown with native milkweed species, *A. incarnata* and *A. tuberosa*. Student-collected data showed that physiologically, A. *curassavica* is a more robust milkweed species compared to the two native milkweed species (Figure 4.4A, Figure C.7) and has improved growth when directly competing with native milkweed species (Figure 4.4B, Figure C.7), especially when grown with *A. tuberosa*. Interestingly, neither native species seemed to benefit or be negatively impacted when grown with the

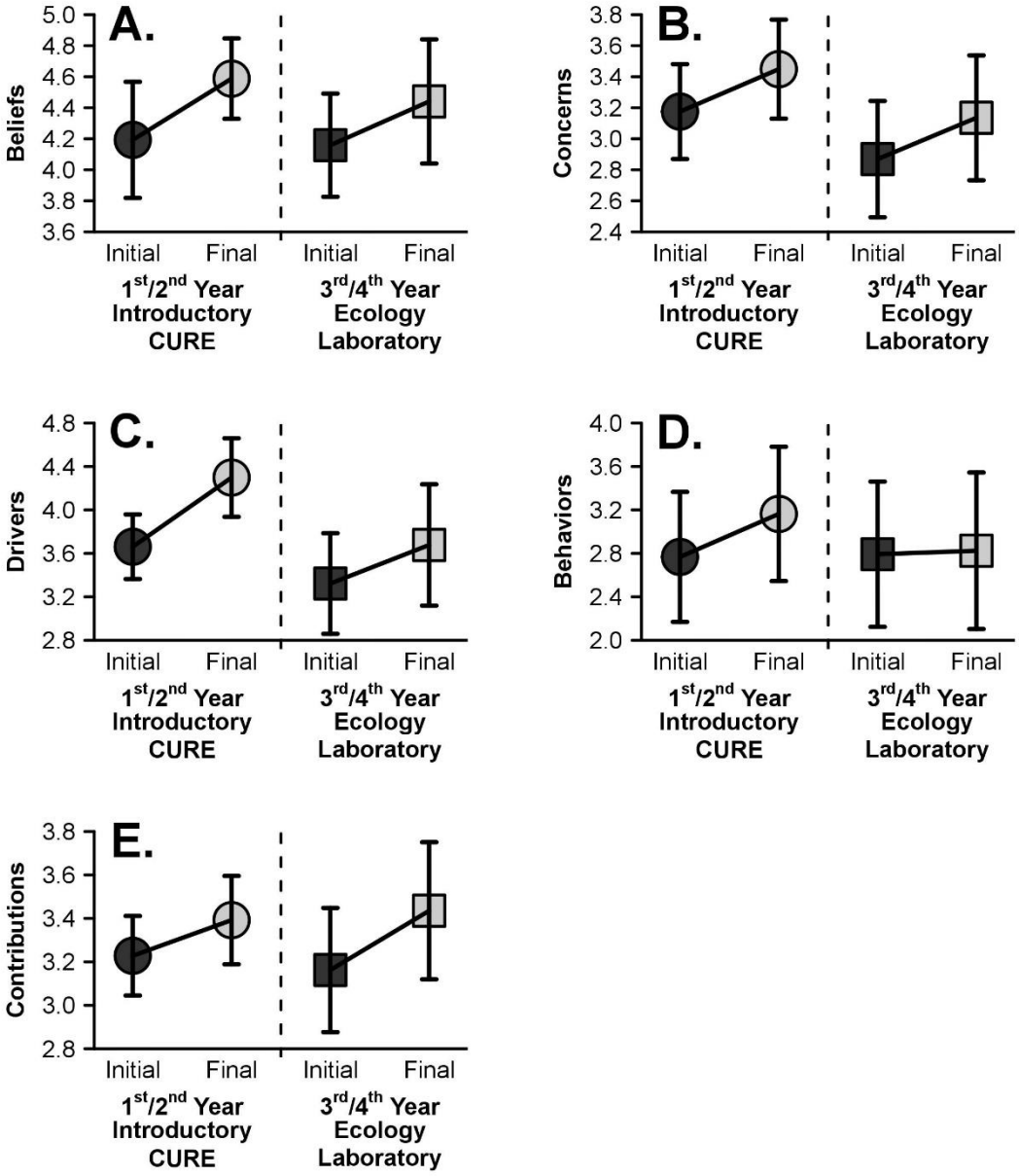

Figure 4.5. Student perceptions of invasive species categorized into beliefs, concerns, drivers, behaviors, and contributions while being exposed to different teaching practices in an early-division CURE laboratory compared to an upper-division, traditional structured Ecology laboratory. Shapes indicate different courses, while shading indicates different sampling times. (A.) The average scores of student's beliefs regarding invasive species, with 95% confidence intervals. (B.) The average scores of students concerns regarding invasive species, with 95% confidence intervals. (C.) The average scores of student's perceptions of the drivers of invasive species, with 95% confidence intervals. (D.) The average scores of student behaviors regarding invasive species, with 95% confidence intervals. (E.) The average scores of student's contributions regarding invasive species, with 95% confidence intervals.

more robust invasive species *A. curassavica* (Figure 4.4C, Figure 4.4D). To this end, *A. curassavica* appears to be a commensalistic competitor with native milkweed species. Commensal competitors benefit from the competitive interaction, while other competing species incur no negative fitness cost from the interaction, and this is often reflected through complex indirect interactions (White *et al.* 2006). Commensalism is notably seen in epiphytic orchids growing in tropical forests, wherein the interaction between orchids and host tress provide valuable community structure (Zotarelli *et al.* 2019). Here, the increased growth of *A. curassavica* with *A. tuberosa* may be driven by *A. tuberosa*'s nutritional investment into a rhizobial rootstalk (Woodson 1954). Plant competition favors plants that can maximize aboveground and belowground physiological traits, with root competition for resources dramatically impacting aboveground plant diversity and community structure (Schenk 2006, Craine and Dybzinski 2013). *A. tuberosa*'s energetic expenditure may require a temporal cost, reducing aboveground growth, and given *A. curassavica*'s developmental propensity, allowing less competition for aboveground resources. While work has shown that increased resource availability and disturbance regimes increase invasive species performance to that of native species (Daehler 2003), often times, high growth rates are necessitated for a species to be a successful invader (Funk and Vitousek 2007, van Kleunen *et al.* 2010). If allowed to grow together for a longer period of time, differences across fitness metrics (as affected by *A. curassavica*'s rapid growth) between the adventive *A. curassavica* and the native milkweed species may be quantified, especially differences influenced by altered growth between the three species that were not apparent given the short duration of growing time (roughly six

weeks). Regardless, *A. curassavica*'s robust overall performance across all fitness metrics underscores its attractiveness as a commercial plant, and if grown with native species rather than in monoculture gardens, may improve milkweed garden structuring.

Campaigns focused on increasing the area of suitable milkweed habitat for monarchs have been underway as monarch populations have experienced historic declines (Belsky and Joshi 2018). For example, milkweed gardens lead to dramatically improved oviposition rates compared to natural sites (Cutting and Tallamy 2015, Belsky and Joshi 2018). Considering that wide-spread, monoculture planting of *A. curassavica* increases parasite prevalence in monarch populations and may form ecological traps for monarchs (Satterfield *et al.* 2015, Faldyn *et al.* 2018), monarch garden design should focus on using a diversity of native milkweed species, rather than relying solely on *A. curassavica*. To maximize effectiveness, best management practices for milkweed gardens need to consider the types of milkweed species being planted. Special consideration should be given to the fact that most invasive plants, many of which were introduced originally for horticultural use in nurseries and gardens, have escaped those original areas (Reichard and White 2001) and that *A. curassavica*, specifically, can set seeds effectively through self-pollination (Ward *et al.* 2012). Together, caution should be used to avoid an over-reliance on *A. curassavica* alone as the sole stand-in substitute host plant for monarch butterflies in lieu of planting a diversity of milkweed species.

CURE: IMPACT ON 1ST AND 2ND YEAR STUDENTS

CURE participation engaged early-division undergraduate students in addressing a relevant ecological topic, exploring a novel scientific question, and increased early-

division CURE student understanding and perceptions of invasive species ecology. Since students in both courses experienced increases in overall perception and understanding of invasive species, and considering that early-division students enrolled in the CURE displayed a marginally greater degree of change in opinion and willingness to modify behaviors about invasive species than upper level students enrolled in the ecology laboratory, the CURE structure benefitted early-division students (Figure 4.5). Early-division student participation in the CURE increased student understanding of the ecological mechanisms underpinning invasive species comparable to that of upperclassmen enrolled in a traditionally structure ecology laboratory, while allowing early-division students access to an authentic research experience (Figure 4.5C). Interestingly, 3rd and 4th year students enrolled in the ecology laboratory displayed marginal improvements in understanding the ecological mechanisms of invasive species (Figure 4.5C), with no differences in understanding of invasive species ecological mechanisms in the initial pre-course survey between the 1st and 2nd undergraduate students and the 3rd and 4th year students. While 3rd and 4th year students were exposed to these topics at multiple points throughout the semester, and specifically in weeks #9- #12 (Figure C.1), little to no change in student understanding may indicate that portions of the laboratory course should be re-evaluated. In general, students in courses designed with traditional-lectures using controlled experiments structure using may not be retaining material as well as students who participate in a more student-centric, active-learning course (Lom 2012). Furthermore, courses that favor the inclusion of engaging, active-learning components rather than lectures alone increase student test scores,

overall academic performance, curricula engagement, course satisfaction, and decrease student failure rates (Armbruster *et al.* 2009, Freeman *et al.* 2014), while also benefiting instructors by fostering communities of education focused on disseminating teaching techniques for high-enrollment STEM course (Tomkin *et al.* 2019).

By combining a moderate amount of lectures with hands-on, active-learning components in the CURE, we were able to make measurable scientific in-roads into 1st and 2nd year undergraduate students understanding and perspective of scientific research, rather than retroactively engaging students in scientific research who are close to graduating. Yet, work shows a fully active-learning classroom environment may not consistently enhance undergraduate achievement across all courses or divisions (Andrews *et al.* 2011, Linneman 2019). Potentially, since most upper-level biology majors at LSU are in career tracks not associated with ecology, those students may be inclined to put less effort in retaining material not relevant to their career interests, especially as they near graduation. While this result may be alarming, more work needs to be done to evaluate if this is an artifact of low response rate and sample size, if upper level students are putting forth diminished effort to retain the material, or conduct a side-by-side comparison between an active-learning, CURE structured upper division ecology course and this traditional lecture-based ecology course. Furthermore, grouping 1st and 2nd year students into a lower-division student classification and 3rd and 4th year into the upper-division classification creates confounded stages for comparison. While one would expect that 3rd and 4th year students, who have taken a greater number of biology courses, would out-perform students who are early in their academic careers (Roth 2015), this additive effect

of classroom exposure could raise the average scores for early-divisional student responses compared to the responses of strictly 1st year students. Splitting these up further would help clarify these confounding factors, dependent on a large sample size. It would also be fruitful to follow these students to see if these effects are maintained across multiple semesters. Furthermore, another important consideration to investigate would be whether or not CURE students are more likely to stay enrolled as biology majors compared to 1st and 2nd year students enrolled in traditional, introductory labs. Finally, because this study was not conducted blindly, potential surveying biases could be mitigated by employing a blind or double-blind survey design. Regardless, CURE students showed a greater increase in understanding the ecological effects of species invasions and were more willing to modify their perceptions and behaviors.

FUTURE MODIFICATIONS AND IMPACTS

Through directly involving students in the scientific process, CURE students addressed a relevant ecological question of how *A. curassavica* competes with native milkweed species, *A. incarnata* and *A. tuberosa*. While *A. curassavica* performs better across all fitness metrics and may provide a short-term solution for ensuring oviposition areas for monarch butterflies, and given *A. curassavica*'s commensalistic growth with native species, consideration for relying less on *A. curassavica* when planting monarch and pollinator gardens should be made. Major pollinator communities (e.g., bumblebees, monarch butterflies) are influenced by the quality of plants in gardens, with garden plant diversity determining the overall success of pollinators, where low plant diversity negatively impacts pollinators (Hulsmann *et al.* 2015, Baker and Potter 2018). Given the

known, potential consequences of widespread planting of *A. curassavica*, reducing migration, increasing disease, and forming ecological traps (Satterfield *et al.* 2015, Faldyn *et al.* 2018), managers and concerned citizens should consider the consequences of widespread planting of *A. curassavica* in pollinator gardens and focus on planting a diversity of milkweed species.

The framework used in this CURE can be modified to a wide-variety of other projects, focusing on an iterative learning of research principles and improving student communication skills (Figure 4.2, Figure 4.3). Given the success of the course with untrained 1st and 2nd year students, we suspect that this course could be modified for high school laboratories, particularly for advanced environmental, biological, or earth sciences courses. Major assignments, rubrics, student designed talks, presentations, assignments, and final posters can be accessed to aid in curricula structuring (Appendix D). Since participation in this CURE increased early division student understanding and perceptions of invasive species to that of an upper-division laboratory, such exposure could have cascading positive implications for the future. When individuals have been exposed to environmental messages at a younger age, environmental awareness increases (McDougle *et al.* 2011). Furthermore, environmentally-conscious individuals often have multi-faceted experiences during formative years with educational experiences listed as a major factor in shaping environmental stewardship (Chawla 1999). By focusing on a critical environmental issue that many students may be unaware of, while providing an opportunity for student ownership, a carefully designed CURE can foster lasting environmental awareness (Bakshi *et al.* 2016). Lastly, these students were directly

exposed to the scientific process from identifying patterns to communicating results (Figure 4.2). Thus, students were being trained on how to do science and not just the common recitation of facts for an exam or laboratory practicum.

In general, CURE participation marginally improved student understanding and perceptions of an ecologically relevant topic compared to a traditional ecology laboratory with the benefit of engaging undergraduate students in addressing a relevant ecological question. By allowing early divisional students to participate in tangible research on relevant environmental topics (e.g., invasive species), CURE participation could have lasting student impacts, leading to greater awareness of environmental issues. Through increased early-divisional student understanding and perceptions of ecological topics, we hope to improve the outlook for this well-known, charismatic insect species that has been experiencing dramatic population declines (Belsky and Joshi 2018). Through this work, our hope is that laboratory coordinators and other STEM educators can implement this CURE framework to both engage and utilize early-divisional students in conducting scientific research. Together, designing courses with these considerations allows educators to foster scientific inroads for early-division undergraduate students while also addressing relevant research topics. Ultimately, CURE courses can mediate the issues associated with traditional undergraduate research pathways while engaging undergraduate students in doing authentic, tangible science.

CHAPTER 5
CONCLUSIONS

My dissertation investigated how climate change will affect species interactions while also developing research opportunities for undergraduate students using the monarch butterfly (*Danaus plexippus*)- milkweed (*Asclepias* sp.) system. Through the use of lab, field, and greenhouse experiments, I focused on the direct and indirect effects of climate change on monarch butterflies, through bottom-up effects as mediated by their milkweed host plants or through top-down pressures from their neogregarine parasite. My dissertation work highlights the importance of understanding the impacts of climate change on species interactions to better understand community-level impacts while also demonstrating how authentic scientific research opportunities can be made accessible to a variety of undergraduate students.

First, in Chapter 2, I assessed the impact of climate on plant-insect interactions. Specifically, I quantified the indirect effects of climate on monarch butterflies as mediated through their milkweed host plants. Using open-top chambers (OTCs) to artificially warm plots to conditions expected by 2080, warmed, OTC-covered and ambient, open plots were placed with either invasive *Asclepias curassavica* or native *A. incarnata*, and monarch larvae. We found that under current climatic conditions, adult monarchs had higher survival and weights when feeding on *A. curassavica*. However, under future conditions, monarchs fared much worse on *A. curassavica*, with the decrease in monarch performance associated with increasing cardenolide concentrations driven by warmer temperatures. Thus, this chapter illustrated that cardenolide concentrations in *A.*

curassavica transitioned from beneficial to detrimental as temperature increased, forming an ecological trap whereby past environmental cues associated with increased fitness to monarchs give misleading information, leading to a negative fitness cost.

This work highlights the importance of understanding how modifications to bottom-up factors (i.e., changes in the quality of a host resource) can have cascading impacts on host performance. Here, we highlight that the impacts of climate change are not limited to direct impacts, but climate change impacts will touch many facets in an ecological community leading to climate change-induced ecological traps. For example, recent development of climate change-induced ecological traps for a wide variety of species such as coastal water marine organisms, African penguins, and for lepidopteran species all underscore the importance of understanding the consequences of species maladaptive behavioral responses to human-induced rapid environmental change (Sih 2013, Van Dyck *et al.* 2015, Sherley *et al.* 2017, Vinagre *et al.* 2018). Furthermore, while this work compliments other studies indicating the impacts of temperature on monarchs and milkweed, it also emphasizes that the overall direction and strength of interactions between any species in an ecological community will be altered regardless of the development of an ecological trap. Considering that for monarch butterflies, the combination of climate change and planting of *A. curassavica* may be exacerbating a developing ecological trap, nurseries should reevaluate milkweed gardening practices. Specifically, the over reliance on *A. curassavica* should be reconsidered, and a diversity of milkweed species should be sold instead. Future work must consider other physiological changes climate change may have on monarch butterflies, specifically female oviposition

preference, and focus on the mechanisms of stress response to temperature and cardenolide production across milkweed species.

Second, in Chapter 3, I empirically assessed how climate change affects host-parasite interactions by employing the monarch butterfly-OE, host-parasite system. I investigated how does elevated temperature and parasite infection affect monarch population dynamics and overall fitness. Warmer temperatures decreased monarch population growth, which was further exacerbated by parasite infection. Sensitivity and elasticity of matrix elements differed depending upon the treatment. For OE-infected and OE-uninfected butterflies, regardless of temperature, almost all populations were sensitive to changes in eclosion from pupae to an adult. Survivorship as a pupa was the most elastic elements regardless of the abiotic or biotic conditions in all monarch populations. In general, monarch survivorship decreased when reared in warmer temperatures (regardless of infection status), but differences in fitness metrics (e.g., development time, weight, melanism, and size across growth stages) between ambient and warm conditions were seen only in OE-infected monarchs. Lastly, OE-infected monarchs reared at increased temperatures are lighter in color and have smaller overall dorsal areas, which can affect individual fitness due to changes in fecundity and/or migration success. To this end, the combination of climate-change induced increases in temperature with simultaneous parasite infection acts as a one-two punch, posing a serious threat to monarch butterflies.

This work illustrates how the direct effects of climate change and simultaneous parasite infection negatively impact a species population growth, development, and

aspects of fitness. The most vulnerable portion of monarch development is pupa survival and successful eclosion into an adult, reinforcing the notion that these stages and transitions should be targeted for management to improve declining monarch populations. Furthermore, given that empirical support for the impacts of climate change on parasite infection and the altered interactions with host species is lacking (Cizauskas *et al.* 2017), this work quantifies these impacts and underscores the importance of using community-modules to tease apart confounded interactions. Specifically, the concomitant stress from increased temperatures and infection decreased survivorship, developmental times, weights, and area for monarchs while increasing melanism. Physiological changes such as these impact rates of predation, adult fecundity, migratory ability, and thermal tolerances. While monarchs displayed some forms of plastic responses to these changes, much like other species, these responses were not enough to compensate for the combination of stressors. Together, the results suggest that both increased temperatures and parasite infection act as major stressors to species, but future studies should specify infection doses apriori and consider using parasite strains of different virulence to fully assess the impacts on a focal species. Furthermore, as more empirical evidence that quantitatively describes these linkages is collected, future meta-analyses should quantify any large-scale trends that may be missed in highly-controlled studies to better inform management and conservation practices.

Third, in Chapter 4, a course-based undergraduate research experience (CURE) using the monarch butterfly (*Danaus plexippus*) and milkweed (*Asclepias*) system was implemented, focusing on an adventive milkweed, *Asclepias curassavica*. Here, I

investigated how does the adventive milkweed, *A. curassavica*, compete with two native milkweed species, *A. incarnata* and *A. tuberosa*. Furthermore, I assessed how CURE participation improved student understanding of an ecologically relevant topic (i.e., invasive species biology) compared to students enrolled in a traditional ecology laboratory by using a hands-on, competition experiment. The factorial competition experiment, carried out over three semesters by CURE students, between the adventive *A. curassavica* and two native milkweed species, *A. incarnata* and *A. tuberosa*, found that *A. curassavica* is a more robust species than native milkweed species across all fitness metrics, with A. curassavica displaying commensal competitive characteristics when competing with native milkweed species. Through pre-course and post-course surveys, it was found that early-division (i.e., 1st/2nd year students) CURE students displayed increases in understanding of invasive species ecology and contributions to invasive species similar to that of upper-division students (3rd/4th year students) in a traditionally structured ecology laboratory. Thus, while early-division CURE students showed equal gains in learning the material to that upper-division students, they had the opportunity to engage and participate in authentic scientific research while doing so.

Ultimately, CURE participation elucidated the competitive interactions of milkweed species and engaged undergraduate students in authentic research, fostering early division student's subject-specific understanding and awareness equitable to that of upper-division students. Here, the adventive milkweed, A. curassavica, was found to be a more robust species than the native milkweed species, *A. incarnata* and *A. tuberosa*, across all fitness metrics. When grown in competition, *A. curassavica*, initially considered

to be an invasive species, did not negatively impact the growth of either native species. Rather, *A. curassavica* grew the best when it competed with the native species, indicating this species may be a commensalist. Often, the direct effects, and especially indirect effects mediated through a secondary species, of competition on invasive species success is difficult to assess and empirical support is lacking (White *et al.* 2006). This work provides insight into these direct and indirect competitive interactions, specifically the role of commensalism in influencing invasion success. Furthermore, early-division CURE students gained authentic research experience and displayed learning gains similar to that of upper-division students. Implementation of CURE-structured courses can promote scientific inroads for early divisional students while also providing effective subject instruction. The CURE designed here can be easily manipulated for a variety of courses, from secondary school biology and environmental sciences courses to introductory biology laboratories at the post-secondary level. To further elucidate commensalism between *A. curassavica*, *A. incarnata*, and *A. tuberosa*, future work should assess similarly designed competition experiments for longer periods of time. Doing so would provide valuable information on life-cycle and phenological effects of competition on these species. Future education studies should emphasize increased response rates, a common problem that was made apparent in the response rate from upper-division students. Furthermore, studies investigating a CURE-style lecture course to that of a traditional lecture course would be fruitful to assess the lecture-component that is the hallmark of traditional university lecture courses. Finally, a study following CURE students throughout their college or university programs to see if these impacts from the

CURE would also lend credence to the CURE framework and implementation in post-secondary education.

Finally, my dissertation research on quantifying the direct and indirect impacts of climate change on monarchs, their parasites, and milkweeds has significant implications for the management of monarch butterfly populations and use as a teaching platform. This works adds empirical support to a growing body of literature underscoring the importance of how climate change will impacts species interactions (see Parmesan (2006) for further reading). Due to the difficulty in teasing apart complex interactions across large ecological communities, this work further highlights the use of small scale community modules to explore the impacts of climate change on ecological communities. From an applied perspective, my dissertation highlights the prevalence at which climate-change induced ecological traps may develop, illustrating to nurseries and concerned citizens to reconsider best gardening practices for milkweed habitat. The overreliance of *A. curassavica* as a host plant for monarchs may be more detrimental to monarch populations as climate change continues, compounded by work indicating this milkweed increases disease prevalence and decreases monarch migratory propensity. Apart from the formation of ecological traps, monarch populations that remain with *A. curassavica* milkweed stands experience increase OE-parasite loads, in particular if those populations with reduced migration (Satterfield *et al.* 2015). Considering that OE-infection and climate change act as one-two-punch negatively affecting monarch performance, planting a diversity of milkweed species (rather than monoculture stands of *A. curassavica*) that can convey adequate fitness benefits to monarchs while also not altering their migratory

behavior would be prudent for management consideration. Lastly, this system provides unique opportunities to engage undergraduate students in authentic research, with applied concepts and results. Continuing to quantify how climate change affects species interactions and how changes in these linkages cascade to alter ecological communities will provide insight on best management practices for a variety of species, novel teaching opportunities, and help inform public policy to best mitigate the ecological impacts of climate change.

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APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 2

FIELD SITE AND EXPERIMENTAL LAYOUT

Figure A.1. Field site and experimental layout at LSU Innovation Park, Baton Rouge, Louisiana, USA.

EFFECT OF OPEN TOP CHAMBERS ON TEMPERATURE

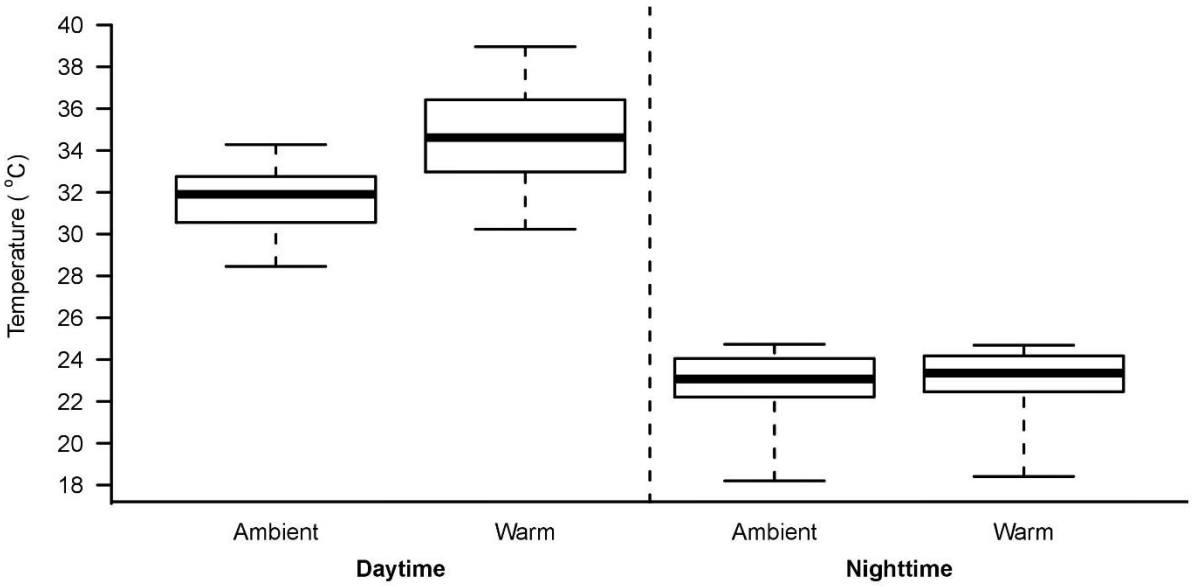

Figure A.2. The passive warming from the open top chambers (OTC) significantly increased ambient temperatures during the daytime. The dark bar in the box-plots represent the average temperature (with quartile ranges on the outer perimeter) between plots with and without an OTC. Here, the OTCs warmed the area within the chamber by roughly 3°C during the daytime and 0.2°C during the nighttime.

CARDENOLIDE COMPOSITION

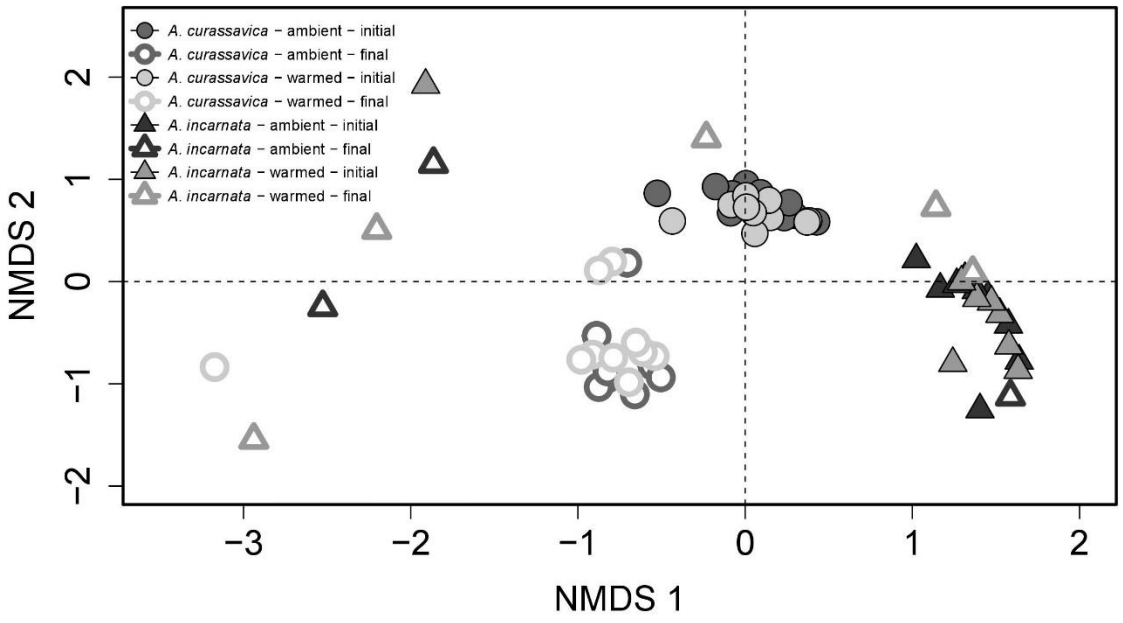

Figure A.3. Shapes represent cardenolide composition of individual milkweed plants placed in ordination space. NMDS axis 1 and NMDS axis 2 aid in visualizing the differences that occur in the composition of the cardenolides produced by both *A. curassavica* and *A. incarnata* between the treatments. From the clustering, cardenolide composition is different between *A. curassavica* and *A. incarnata* and changes during the two weeks between the initial and final plant trait measurements. Together, cardenolide composition reflects the interaction between milkweed plant species and sampling date.

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 3

FIELD SITE, EXPERIMENTAL LAYOUT, AND OE-INFECTION ASSESSMENT

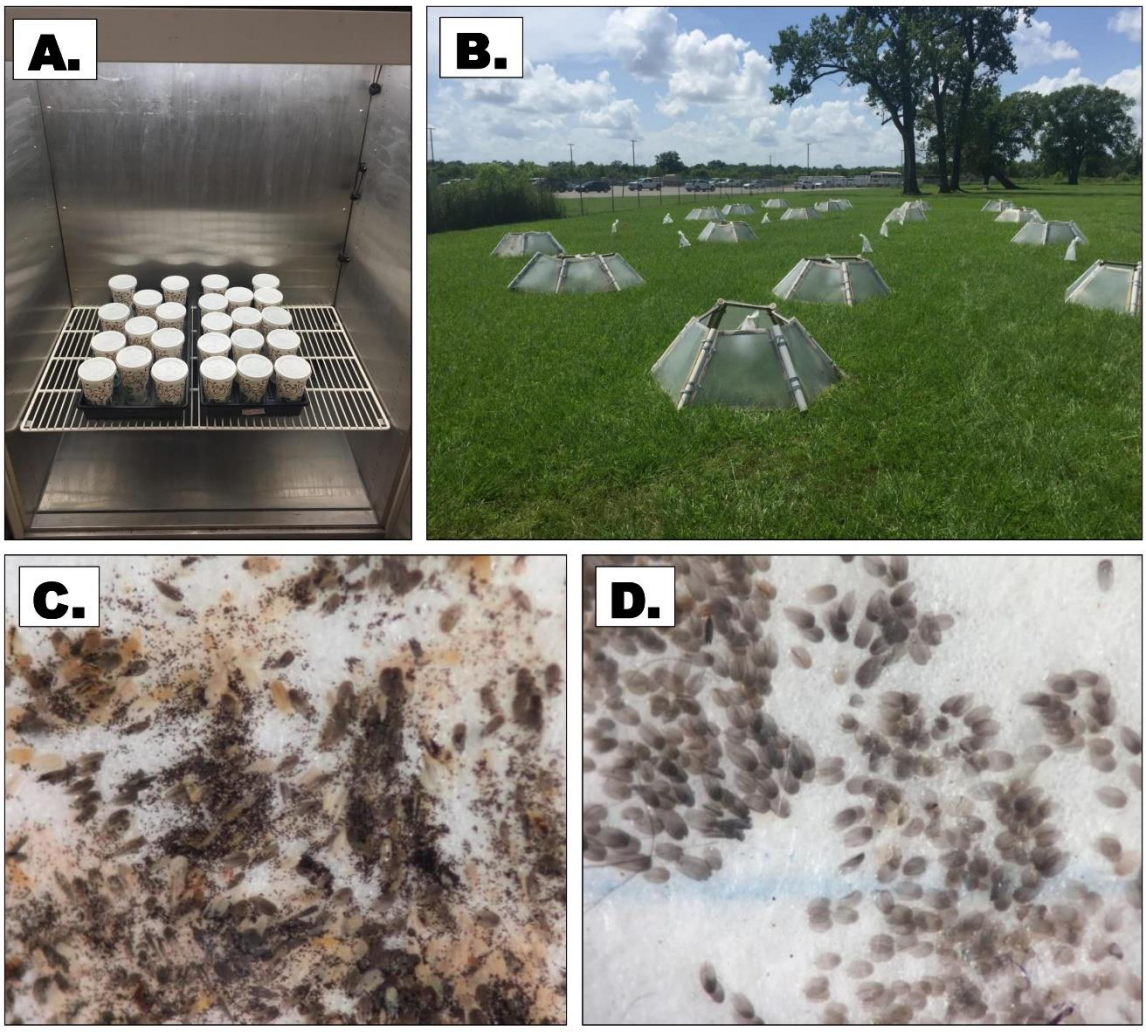

Figure B.1. Pictures highlighting the work performed throughout the experiment. (A.) Laboratory layout of the OE-infected monarch butterflies reared in the lab in the LSU Life Sciences Building, Baton Rouge, LA, USA. OE-uninfected experimentation was carried out using a similar design. (B.) Field layout using ambient (open) plots and OTCs to warm plots of OE-infected monarch butterflies, performed at LSU Innovation Park, Baton Rouge, Louisiana, USA. (C.) OE-infection scores were assessed from spore counts, where OE-spores look like "dust'", or small football-shaped cells. OE-spore counts were performed post-experimentation, and the spore load score shown here indicates a heavy OE-infection from a monarch butterfly that survived ambient temperature conditions. (D.) Here, a monarch butterfly that survived the warmed treatment conditions reflects a zero to low OE-spore load, indicating potential clearing of OE-infection if survival under warmed conditions is possible.

EFFECT OF OPEN TOP CHAMBERS ON TEMPERATURE AND HUMIDITY

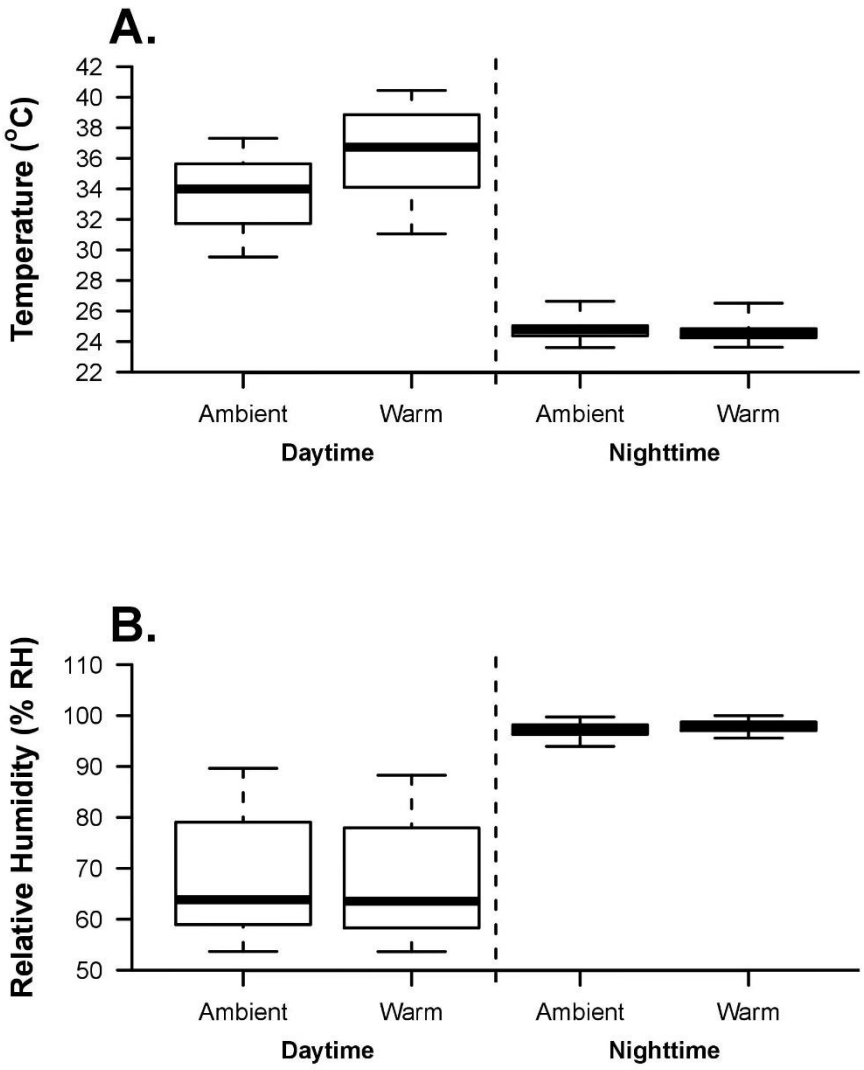

Figure B.2. The effect of open top chambers (OTCs) on plot temperature and relative humidity. (A.) The passive warming from the OTCs significantly increased ambient temperatures during the daytime, and there was dramatic day-to-day variation in temperature. The dark bar in the box-plots represent the average temperature (with quartile ranges on the outer perimeter) between plots with and without an OTC. Here, the OTCs warmed the area within the chamber by roughly 2.5°C during the daytime, and had little effect on nighttime temperatures. (B.) The passive OTCs did not affect relative humidity levels compared to open, ambient plots, although there was dramatic day-to-day variation in humidity. The dark bar in the box-plots represent the average relative humidity (with quartile ranges on the outer perimeter) between plots with and without an OTC.

OE-INFECTED MONARCH FITNESS METRICS

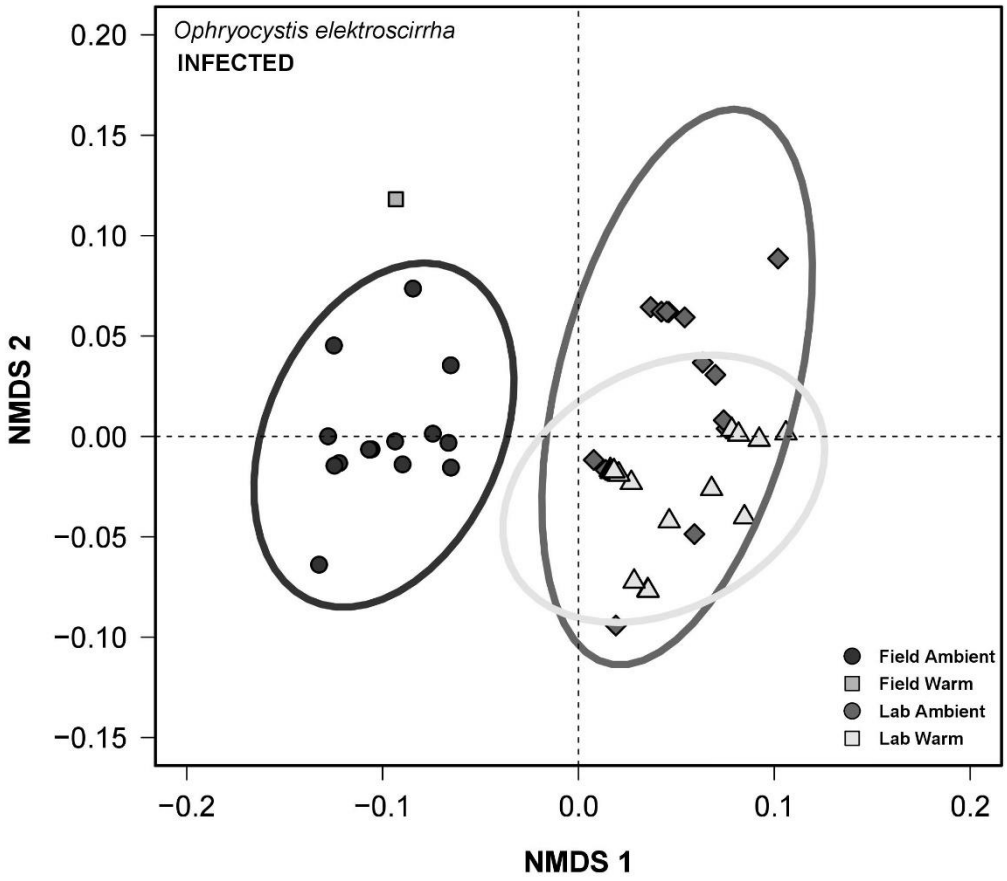

Figure B.3. Shapes represent individual monarch butterflies placed in ordination space. NMDS axis 1 and NMDS axis 2 aid in visualizing the differences that occur between *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Ellipses represent 95% confidence interval areas around a centroid point relative to the ordination clustering. Dark colors represent ambient conditions; light colors represent warm conditions. Separate clustering between ambient or warmed treatment conditions (PerMANOVA temperature condition, stress=0.11 after 20 runs, $F_{1,46}$=58.35, p=0.001) and field and lab experimental sites (PerMANOVA experimental site, stress=0.11 after 20 runs, $F_{1,46}$=55.95, p=0.001) indicates distinct differences across fitness metrics. Temperature treatment condition has a marginally greater effect on fitness metrics than experimental site location. From the distinct clustering of ordination points, it is clear temperature treatment conditions impacts fitness metrics, across experimental sites PerMANOVA temperature condition and experimental site interaction, stress=0.11 after 20 runs, $F_{1,46}$=4.45, p=0.037.

STABLE STAGE DISTRIBUTIONS

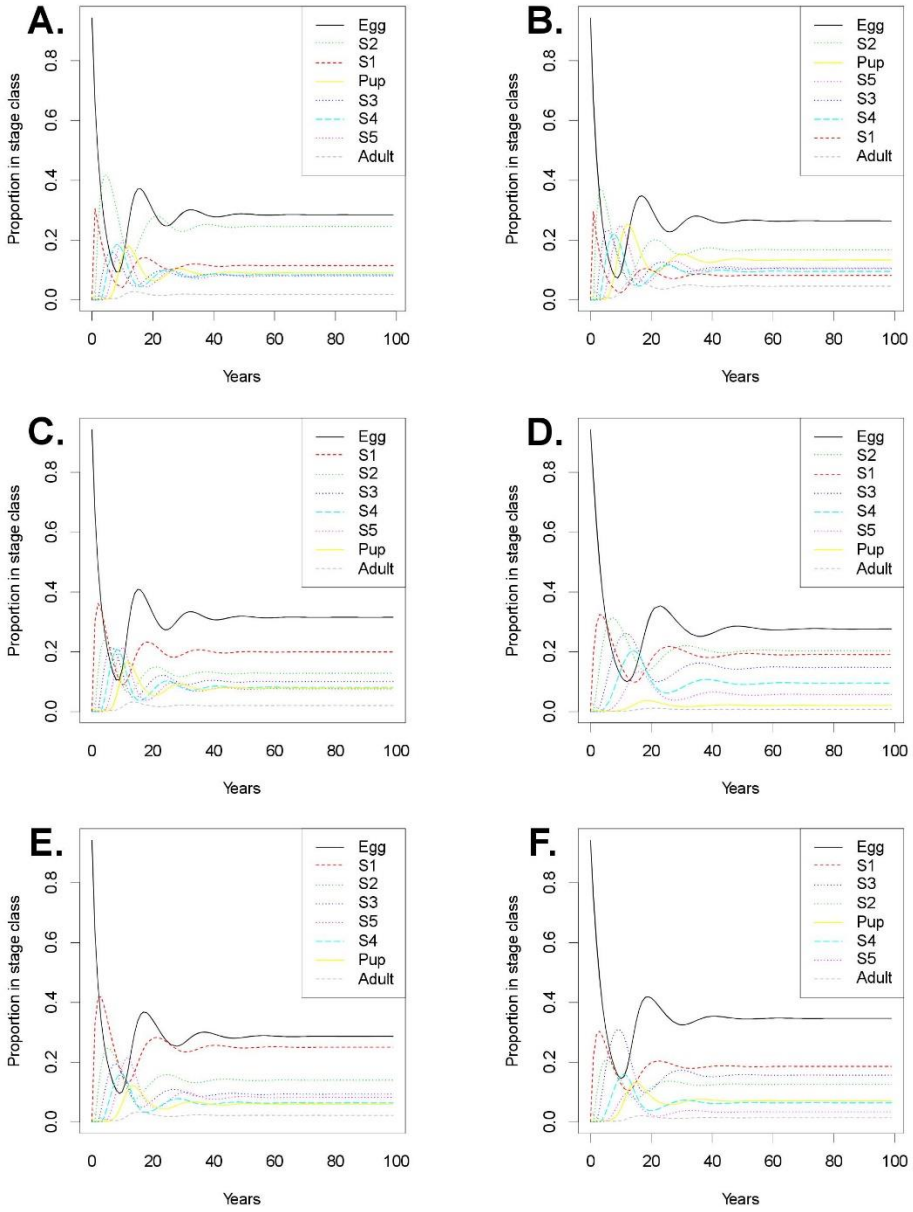

Figure B.4. Stable stage distributions for *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. (A.) Stable stage distribution for OE-infected, lab reared monarchs under ambient temperature conditions. (B.) Stable stage distribution for OE-infected, lab reared monarchs under warm temperature conditions. (C.) Stable stage distribution for OE-infected, field reared monarchs under ambient temperature conditions. (D.) Stable stage distribution for OE-infected, field reared monarchs under warm temperature conditions. (E.) Stable stage distribution for OE-uninfected, lab reared monarchs under ambient temperature conditions. (F.) Stable stage distribution for OE-uninfected, lab reared monarchs under warm temperature conditions.

OE-INFECTED AND OE-UNINFECTED MONARCH BUTTERFLY VITAL RATES

Table B.5. Vital rates for *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites.

Monarch Life History Stages	Vital Rate Descriptions	OE Infected Lab Ambient	OE Infected Lab Warm	OE Infected Field Ambient	OE Infected Field Warm	OE Uninfected Lab Ambient	OE Uninfected Lab Warm
S_{11}	Probability of surviving as an egg	Ambient	0.51	0.63	0.51	0.63	0.51
G_{21}	Probability of growing into a 1st instar larva	Warm	0.47	0.34	0.47	0.34	0.47
S_{22}	Probability of surviving as a 1st instar larva	Ambient	0.00	0.00	0.45	0.55	0.60
G_{32}	Probability of growing into a 2nd instar larva	Warm	1.00	1.00	0.55	0.45	0.38
S_{33}	Probability of surviving as a 2nd instar larva	Ambient	0.71	0.60	0.34	0.62	0.47
G_{43}	Probability of growing into a 3rd instar larva	Ambient	0.29	0.40	0.59	0.27	0.44
S_{44}	Probability of surviving as a 3rd instar larva	Warm	0.31	0.44	0.45	0.67	0.49
G_{54}	Probability of growing into a 4th instar larva	Ambient	0.69	0.56	0.55	0.26	0.43
S_{55}	Probability of surviving as a 4th instar larva	Warm	0.48	0.48	0.52	0.64	0.52
G_{65}	Probability of growing into a 5th instar larva	Ambient	0.52	0.52	0.48	0.20	0.46
S_{66}	Probability of surviving as a 5th instar larva	Ambient	0.63	0.62	0.68	0.71	0.78
G_{76}	Probability of pupating	Warm	0.37	0.37	0.32	0.06	0.21
S_{77}	Probability of surviving as a pupa	Ambient	0.85	0.79	0.89	0.88	0.86
G_{87}	Probability of eclosing into an adult	Warm	0.08	0.13	0.11	0.13	0.14
F_{18}	Number of eggs oviposited	Ambient	10.35	2.63	10.57	14.80	8.04
S_{88}	Probability of surviving as an adult	Warm	0.76	0.70	0.76	0.70	0.76

SENSITIVITY VALUES

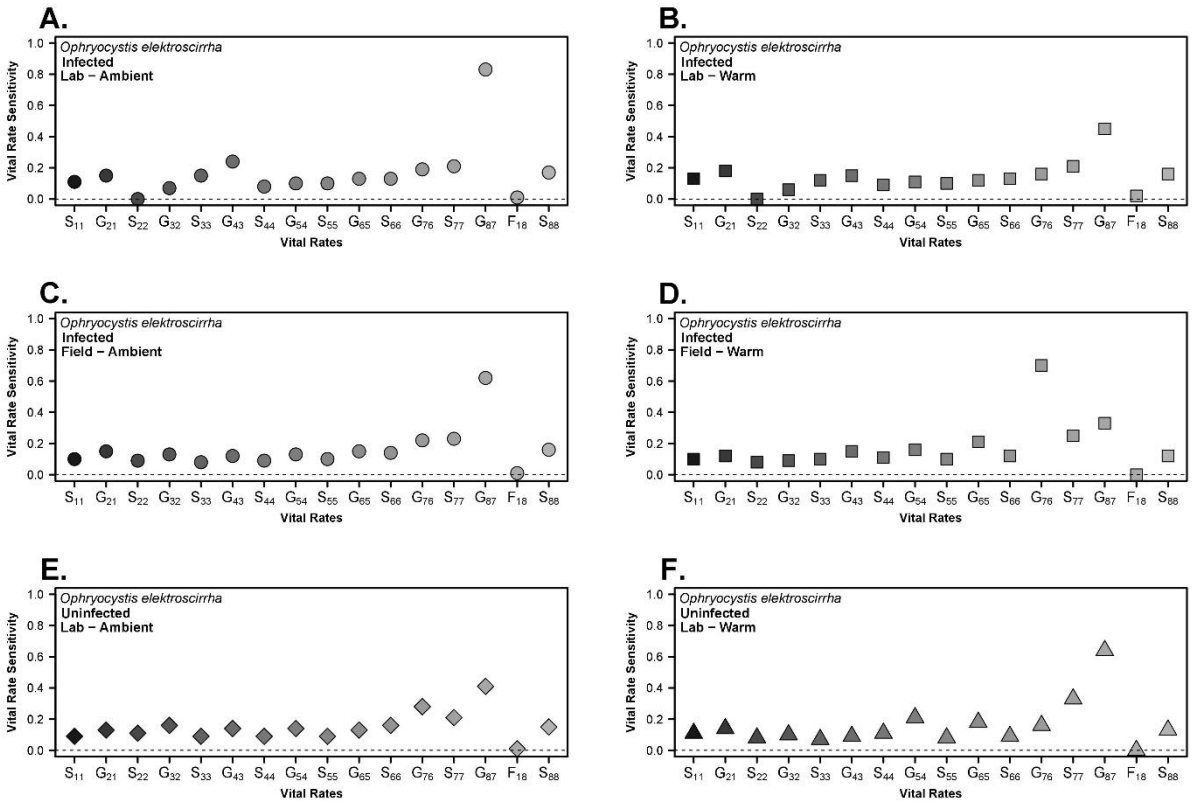

Figure B.5. Sensitivity values for each vital element across the life histories of *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Colors represent different vital rates. Notes the that rate G_{87}, the probability of eclosing into an adult from a pupa, is the most consistent vital rate with the highest sensitivity value. (A.) Sensitivity values for OE-infected, lab reared monarchs under ambient temperature conditions. (B.) Sensitivity values for OE-infected, lab reared monarchs under warm temperature conditions. (C.) Sensitivity values for OE-infected, field reared monarchs under ambient temperature conditions. (D.) Sensitivity values for OE-infected, field reared monarchs under warm temperature conditions. (E.) Sensitivity values for OE-uninfected, lab reared monarchs under ambient temperature conditions. (F.) Sensitivity values for OE-uninfected, lab reared monarchs under warm temperature conditions.

ELASTICITY VALUES

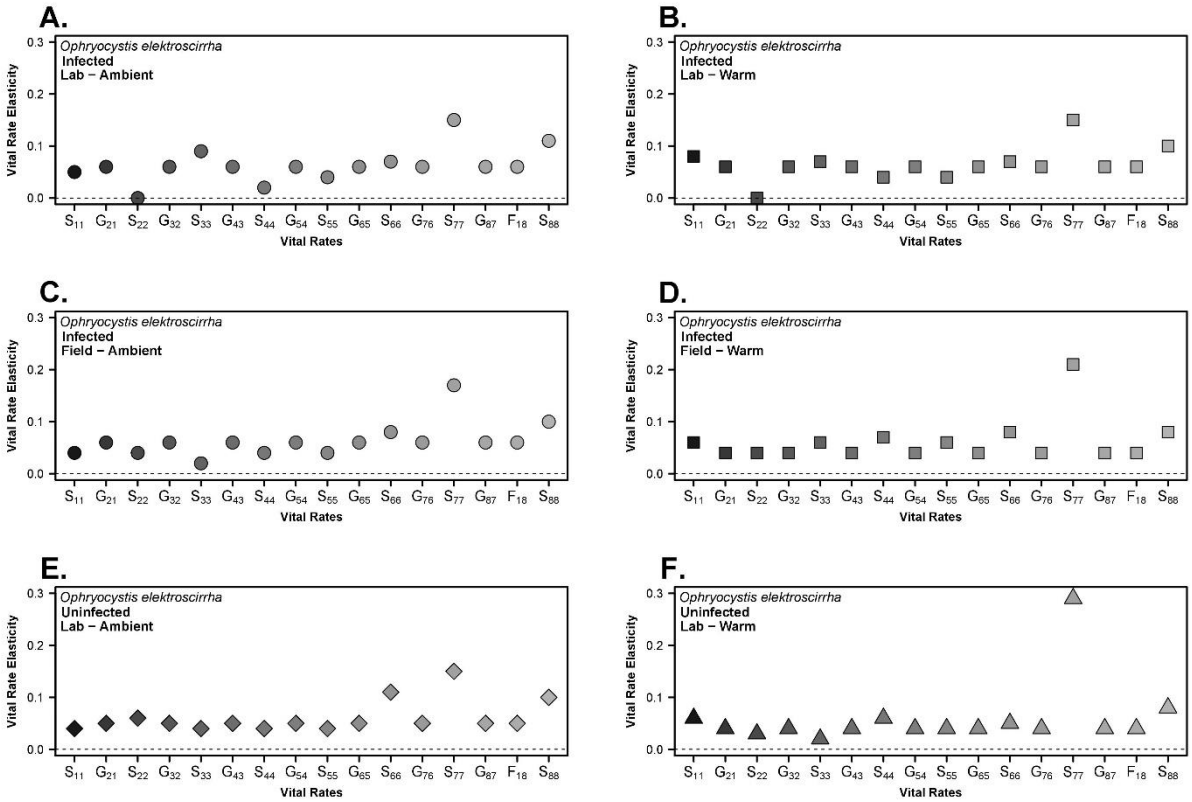

Figure B.6. Elasticity values for each vital element across the life histories of *Ophryocystis elektroscirrha* infected and uninfected monarch butterflies reared under warm or ambient conditions between lab and field experimental sites. Colors represent different vital rates. Notes the that rate S_{77}, the probability of surviving as a pupa, is the most consistent vital rate with the highest elasticity value. (A.) Elasticity values for OE-infected, lab reared monarchs under ambient temperature conditions. (B.) Elasticity values for OE-infected, lab reared monarchs under warm temperature conditions. (C.) Elasticity values for OE-infected, field reared monarchs under ambient temperature conditions. (D.) Elasticity values for OE-infected, field reared monarchs under warm temperature conditions. (E.) Elasticity values for OE-uninfected, lab reared monarchs under ambient temperature conditions. (F.) Elasticity values for OE-uninfected, lab reared monarchs under warm temperature conditions.

OE-INFECTED MONARCH FITNESS METRICS

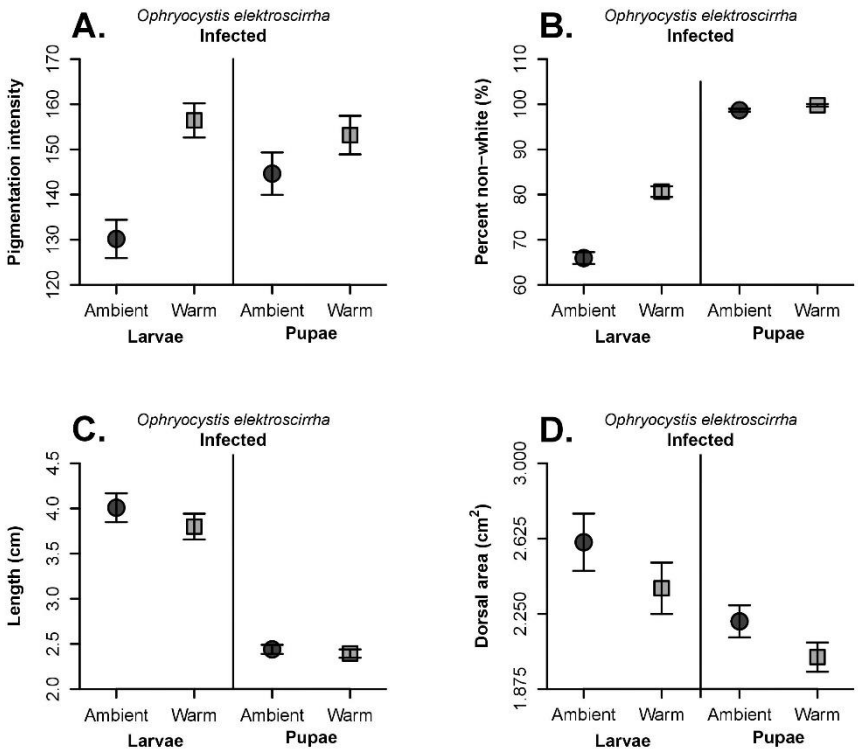

Figure B.7. The average pigmentation intensity, percent non-white, length, and dorsal area of *Ophryocystis elektroscirrha* infected monarch butterfly larvae and pupae reared under warm or ambient conditions between lab and field experimental sites. Dark colors represent ambient conditions; light colors represent warm conditions. (A.) The average pigmentation intensity of monarch larvae and pupae, with 95% confidence intervals. Note the increase in pigmentation intensity (e.g., became lighter colored, overall) in larvae in warmed treatment conditions. (B.) The average percent non-white of monarch larvae and pupae, with 95% confidence intervals. Note the increase in larvae non-white area (e.g., increase in larval yellow banding) under warm conditions. (C.) The average length of monarch larvae and pupae, with 95% confidence intervals. (D.) The average dorsal area of monarch larvae and pupae, with 95% confidence intervals. Note the decrease in pupae area when reared under warmed conditions.

OE-UNINFECTED MONARCH FITNESS METRICS

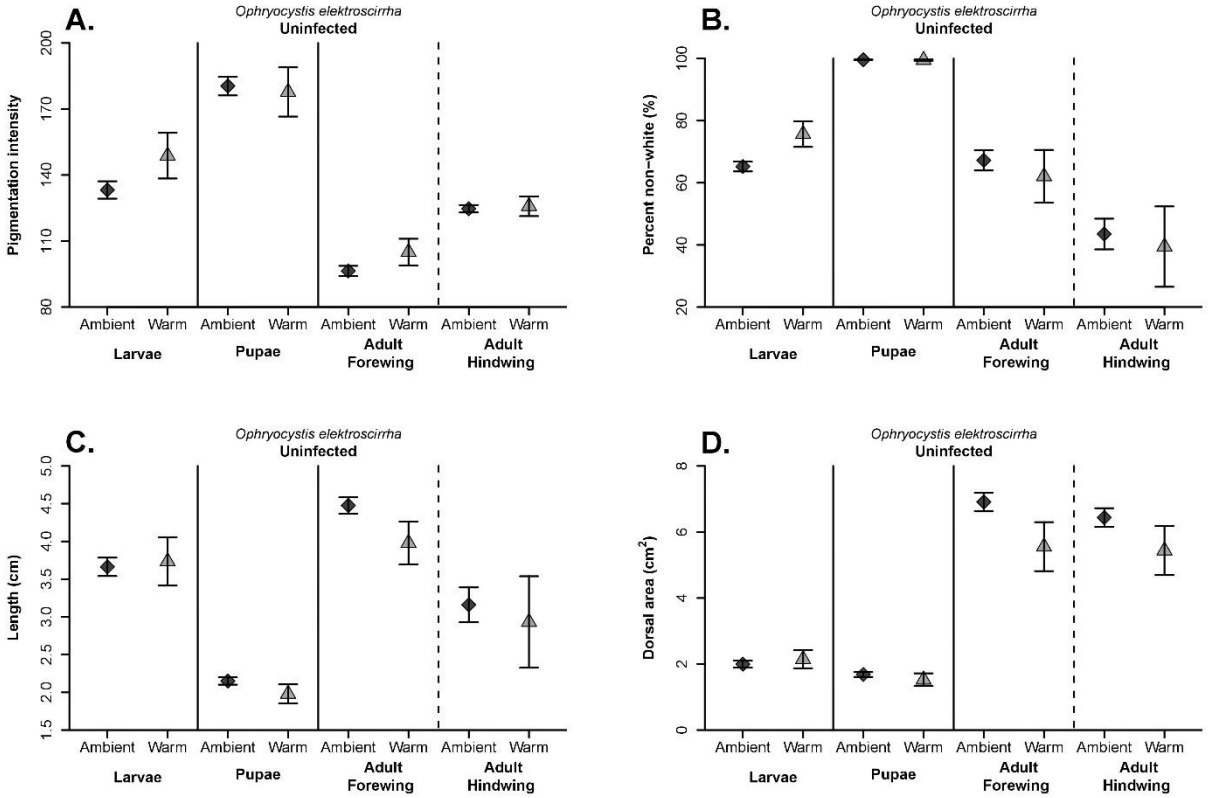

Figure B.8. The average pigmentation intensity, percent non-white, length, and dorsal area of *Ophryocystis elektroscirrha* uninfected monarch butterfly larvae, pupae, and adults (i.e., adult forewings and hindwings) reared under warm or ambient conditions between lab and field experimental sites. Dark colors represent ambient conditions; light colors represent warm conditions. (A.) The average pigmentation intensity of monarch larvae, pupae, and adults (i.e., adult forewings and hindwings), with 95% confidence intervals. (B.) The average percent non-white of monarch larvae, pupae, and adults (i.e., adult forewings and hindwings) with 95% confidence intervals (C.) The average length of monarch larvae, pupae, and adults (i.e., adult forewings and hindwings) with 95% confidence intervals. Note the decrease in adult forewing length under warmed conditions. (D.) The average dorsal area of monarch larvae, pupae, and adults (i.e., adult forewings and hindwings) with 95% confidence intervals.

APPENDIX C. SUPPLEMENTARY MATERIAL FOR CHAPTER 4

UNDERGRADUATE CURE STUDENT AUTHORS

Table C.1. List of LSU undergraduate student authors enrolled in the CURE course.

Resan Abunaser
Tochukwu Agwu
Ahmad Amous
Bayleigh Anders
Rachael Ballou
Austin Barnes
Christian Baskerville
Parker Belaire
Kate Bernard
Tierra Blair
Deanna Bourgeois
Blake Bramley
Justine Brewer
Grace Bridges
Adam Broussard
Clint Brownell
Madeline Burk
Sol Calderon
Jordyn Carmouche
Beatrice Ceron
Seth Chapman
Logan Clement
Sydney Cockburn
Alana Colligan
Ambernecia Cooksey
Kathrine Costanza
Nicholas Crawford
Onesty Culpepper
Anthony Dargin
Rachel Dawson
Dana Deriancho
Amanda Doell
Grant Emfinger
Sissel Erickson
Gabrielle Fantroy
Angele Fels
Alexis Finch
Jessica Francisco
Katie Gatewood
Nathan Gill
Ian Gray
Monica Gros
Kevin Gueniot
Hannah Guimbellot
Gavin Gusler
Kaitlyn Gustinger
Matthew Hailey
Charitey Hall
Brayton Hammes
Ruby Harriford
Faith Harris
Arianna Hatcher
Caroline Haydel
Madeline Haydel
Emily Heath
Diana Hernandez
Jared Hicks
Reagan Hill
Tamia Hutchinson
Elaine Huynh
Peter Issa
Shatara Jackson
Samantha Jackson
Hayley Jackson
Bailey Jarreau
Zoha Javaid
Peyton Jeffcoat
Gabriela Jerez
Quintrele Jones
Logan Jordan
Hannah Keller
Reid Kern
Annum Khan
Evelyn Klein
Olivia Kluchka
Theresa LaForge
Colin Landry
Mya Leake
Rachel Ledet
Schyler Lee
Brea Leslie
Justin Lorio
Carley Loup
Tynia Madison
Nicole Maisano
Makenzie Marshall
Karson Matherne
Birch Matus
Kiana McClendon
Robert McDuff
Sean McGoey
Madisyn McLean
Mary Melancon
Zachary Mendheim
Victor Morales
Heather Moyer
Katelynn Munster
Kieu Ngo
Brandon Nguyen
Anna Nikonenko
Emily Obman
Emily O'Brien
Raymond Ohler
Evan Olsen
Emily Orgeron
Paul Orr
Anthony Parker
Connor Parrino
Megna Patel
Destiny Phanor
Hannah Poirrier
Katherine Pouliot
Eva Pouncey
Nathan Randazzo
Ryan Redmann
Joseph Reynolds
Zoe Richard
Sarah Riviere
Ethan Rocha
Gabrielle Rodemann
Bill Romero
Caleb Romig
Elise Schuyten
Allison Seward
Katie Shaw
Demarcus Shepherd
Shale Silva
Blair Simon
Sharandeep Singh
Sydney Small
Brooke Smith
Keaton Srigley
Sadie Stanchec
Elizabeth Stewart
Megan Stewart
Stephanie Tassin
Madeline Thomas
Carolyn Tran
Vu Tran
Valerie Traylor
Rachel Trimble
Phucphil Trinh
Joshua Tuminello
Briana Tumminello
Elizabeth Turnage
Maria Vargas
Michelle Vetter
Renee Viator
Marshall Vick
Payton Vicknair
Aubrey Vidal
John Waldvogel
Mikaela Walters
Taylor Washington
Symantha Weaver
Taylor Whitworth
Macey Williams
Jennifer Windham
Jailyn Woods
Heidi Wright

ECOLOGY LABORATORY SYLLABUS

Week	Topic	Assignment Due
1	Course Introduction; Data Analysis (computer lab)	
2	Dispersal I (lab)	Statistics Homework Assignment (5%)
3	Disperal II (field)	
4	Life Tables I (field)	
5	Life Tables II (lab)	Dispersal Lab Report (10%)
6	Habitat Variables I (field)	Life Tables Homework Due (5%)
7	Habitat Variables II (field)	
8	Habitat Variables III (field)	
9	Invasive Species I (field)	
10	Invasive Secies II (field)	Habitat Variables Lab Report (20%)
11	Invasive Species III (field)	
12	Presentation Day/ review (lab)	Invasive Species Presentation (15%)
13	Final Laboratory Exam (computer lab)	Final Exam (20%); Invasive Species Proposal (10%)

Figure C.1. Syllabus for the upper-division Ecology laboratory course during the Spring 2018 semester.

LOWER-DIVISION CURE COMPARED TO UPPER-DIVISION ECOLOGY LECTURE

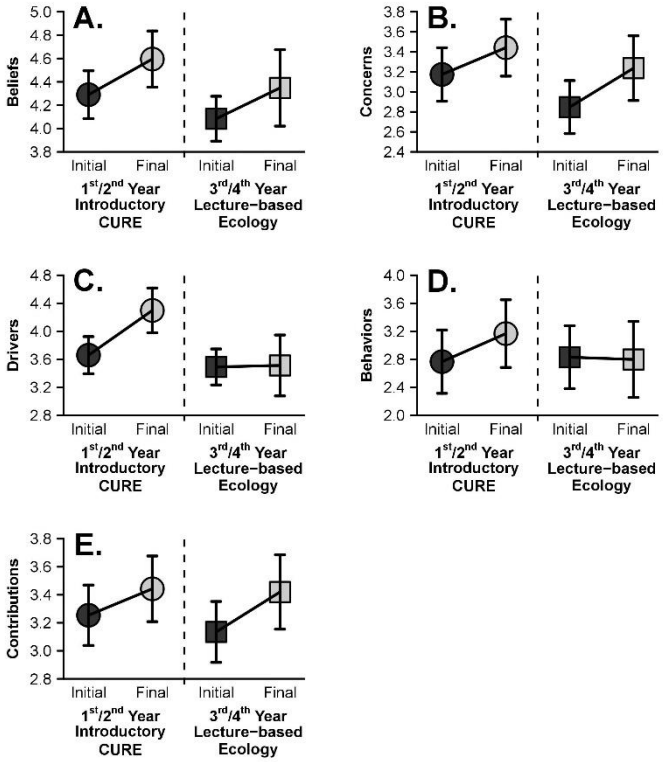

Figure C.2. Student perceptions of invasive species categorized into beliefs, concerns, drivers, behaviors, and contributions while being exposed to different teaching practices in an early-division CURE laboratory compared to an upper-division, traditional structured Ecology lecture. Shapes indicate different courses, while colors indicate different sampling times. Overall, early-division undergraduate CURE students experienced concomitant changes in perceptions towards invasive species similar to upper-division ecology laboratory students. There were no differences in initial survey responses between early-division undergraduate CURE students compared to upper-division ecology laboratory students. (A.) Participation in either course led to a 7% increase in student beliefs about the negative effects of invasive species (trial, $F_{1,34.8}=6.12$, $p=0.0184$). (B.) Participation in either course increased student concerns about invasive species by 11% (trial, $F_{1,25.4}=20.01$, $p<0.0001$). (C.) The interaction of course enrollment and participation in the course throughout the semester led to a 17% increase in student understanding of the mechanisms that drive invasive species ecology for early-division undergraduate CURE students compared to upper-division ecology laboratory students, who showed no change in ecological understanding throughout the semester (course by trial interaction, $F_{1,49}=4.23$, $p=0.0452$. (D.) Early-division undergraduate CURE students showed a 14% increase in willingness to change behaviors that contribute to invasive species spread compared to upper-division ecology laboratory students (course by trial interaction, $F_{1,38.3}=4.11$, $p=0.0495$). (E.) Enrollment in either course led to an 8% increase in student understanding of their contributions to invasive species spread and the impact invasive species can have on their personal lives (trial, $F_{1,29.2}=13.35$, $p<0.0001$).

EXPERIMENTAL LAYOUT AND CURE STUDENT ENGAGEMENT

Figure C.3. Pictures highlighting the work carried out in the CURE course. (A.) Layout of the *Asclepias* sp. competition treatments in the LSU Greenhouses, AgCenter Horticulture Center, Baton Rouge, LA, USA. (B.) Undergraduate CURE students collected milkweed plants metrics in small groups. (C.) A group of undergraduate students collecting leaf toughness measurements using rip-o-meters. (D.) Undergraduate students counting the number of leaves on *A. curassavica* plants. (E.) CURE students presenting their research and completed poster at the CURE symposium. (F.) CURE symposium attendance and audience interaction with lower-division CURE students.

MILKWEED FITNESS METRICS

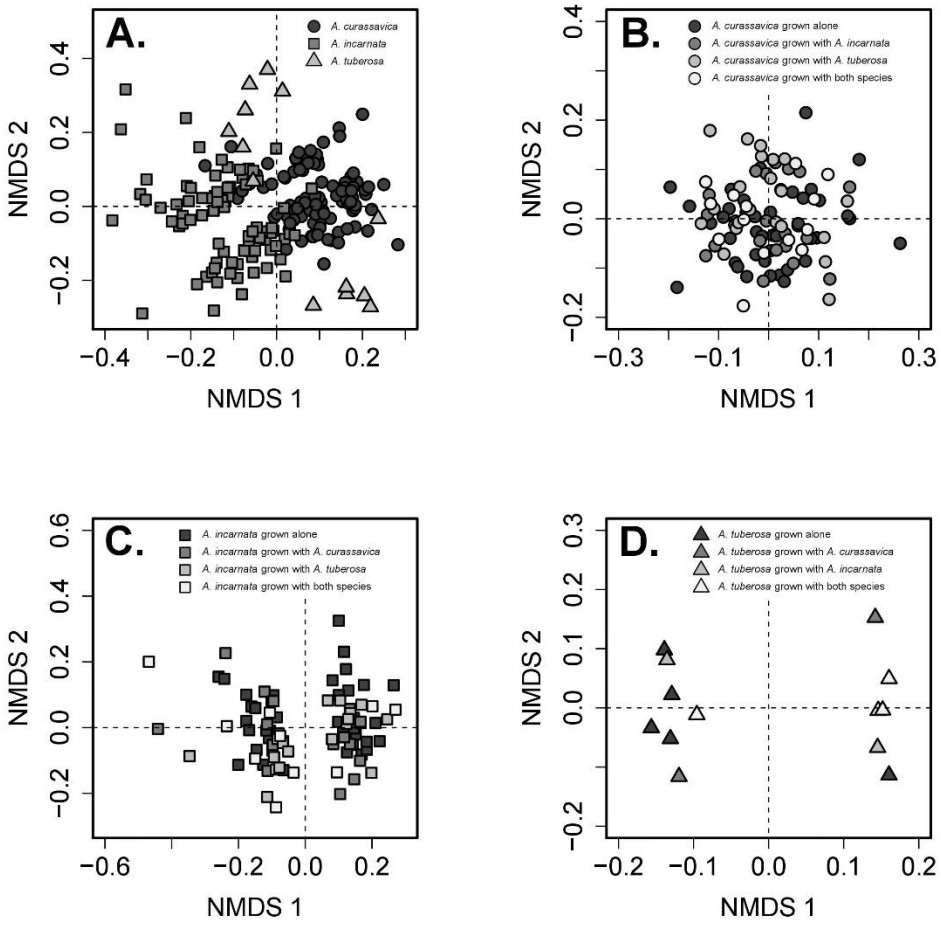

Figure C.4. Shapes represent different milkweed plant species, or different competition treatments, placed in ordination space for milkweed data collected during the Spring 2018 semester. Collected data includes the number of stems, number of leaves, plant height, average stem length, number of flowers (only for *A. curassavica*), latex exudation, aboveground biomass, belowground biomass, and leaf toughness. (A.) The three milkweed species displayed differences when considering all fitness metrics (PerMANOVA, stress=0.19 after 20 runs, $F_{5,189}$=39.147, p=0.001). From the distinct clustering of species ordination points, it is clear the three species display distinct morphological traits. (B.) Colors represent *A. curassavica* plants grown in competition. Individual *A. curassavica* plants grown with native milkweeds experienced no differences across plant traits (PerMANOVA, stress=0.25 after 20 runs, $F_{3,94}$=0.829, p=0.54) (C.) Colors represent *A. incarnata* plants grown in competition. Individual *A. incarnata* plants grown with the invasive and native milkweed experienced no differences across plant traits (PerMANOVA, stress=0.16 after 20 runs, $F_{3,79}$=1.469, p=0.17) (D.) Colors represent *A. tuberosa* plants grown in competition. Individual *A. tuberosa* plants grown with the invasive and native milkweed experienced no differences across plant traits (PerMANOVA, stress=0.1 after 20 runs, $F_{3,9}$=1.703, p=0.16).

MILKWEED SURVIVAL

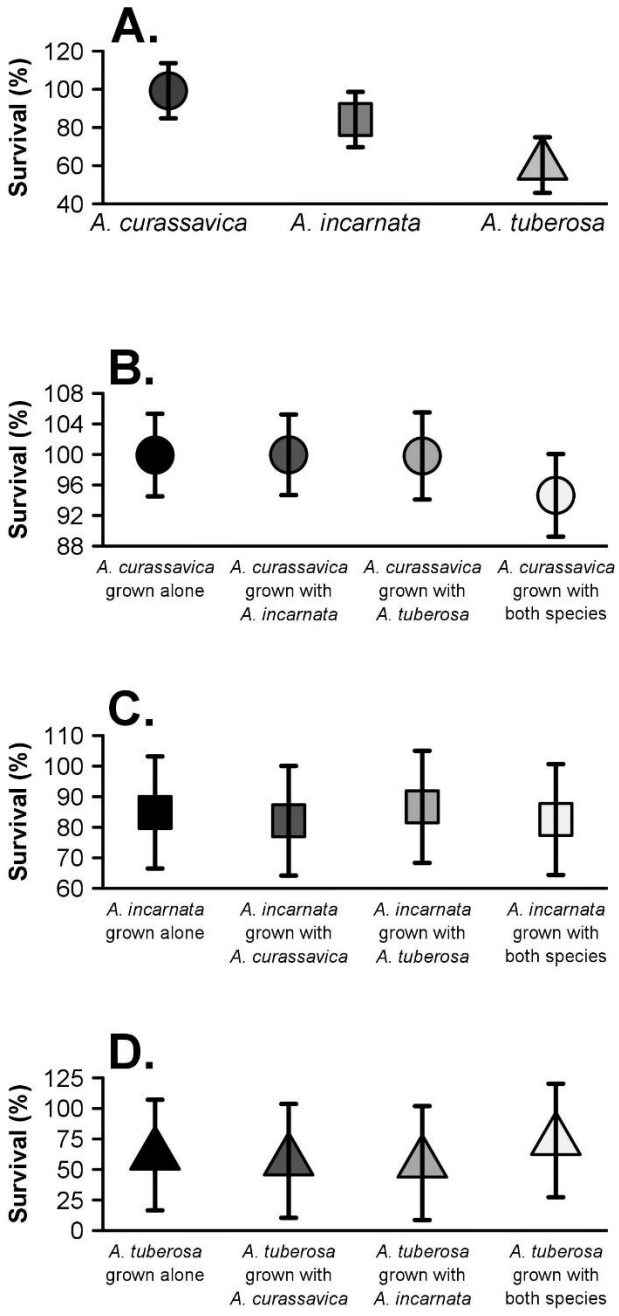

Figure C.5. The survival of individual milkweed plants across all semesters. (A.) The average survival of each milkweed species, with 95% confidence intervals. (B.) The average survival of *A. curassavica* plants grown in competition, with 95% confidence intervals. (C.) The average survival of *A. incarnata* plants grown in competition, with 95% confidence intervals. (D.) The average survival of *A. tuberosa* plants grown in competition, with 95% confidence intervals. *A. curassavica* individuals survived in greater proportion compared to *A. incarnata* and *A. tuberosa*.

MILKWEED FITNESS METRICS ACROSS ALL SPECIES

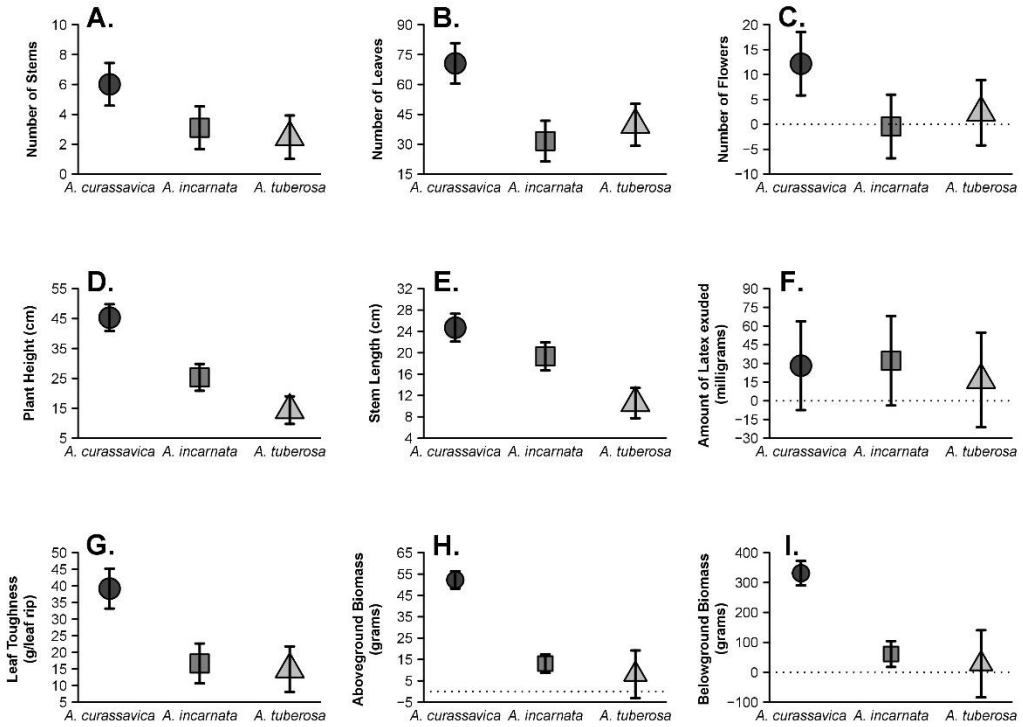

Figure C.6. Fitness metrics between the three milkweed species across all semesters. (A.) The average number of stems, with 95% confidence intervals. *A. curassavica* produced the most stems. (B.) The average number of leaves, with 95% confidence intervals. *A. curassavica* produced the most leaves. (C.) The average number of flowers, with 95% confidence intervals. *A. curassavica* produced the most flowers. (D.) The average height of each milkweed species, with 95% confidence intervals. *A. curassavica* plants grew the tallest. (E.) The average stem length, with 95% confidence intervals. *A. curassavica* produced the longest stems. (F.) The average amount of latex exuded, with 95% confidence intervals. *A. curassavica* exuded marginally more latex. (G.) The average leaf toughness, with 95% confidence intervals. *A. curassavica* produced the toughest leaves. (H.) The average aboveground biomass, with 95% confidence intervals. *A. curassavica* produced the most aboveground biomass. (I.) The average belowground biomass, with 95% confidence intervals. *A. curassavica* produced the most belowground biomass.

A. CURASSAVICA FITNESS METRICS

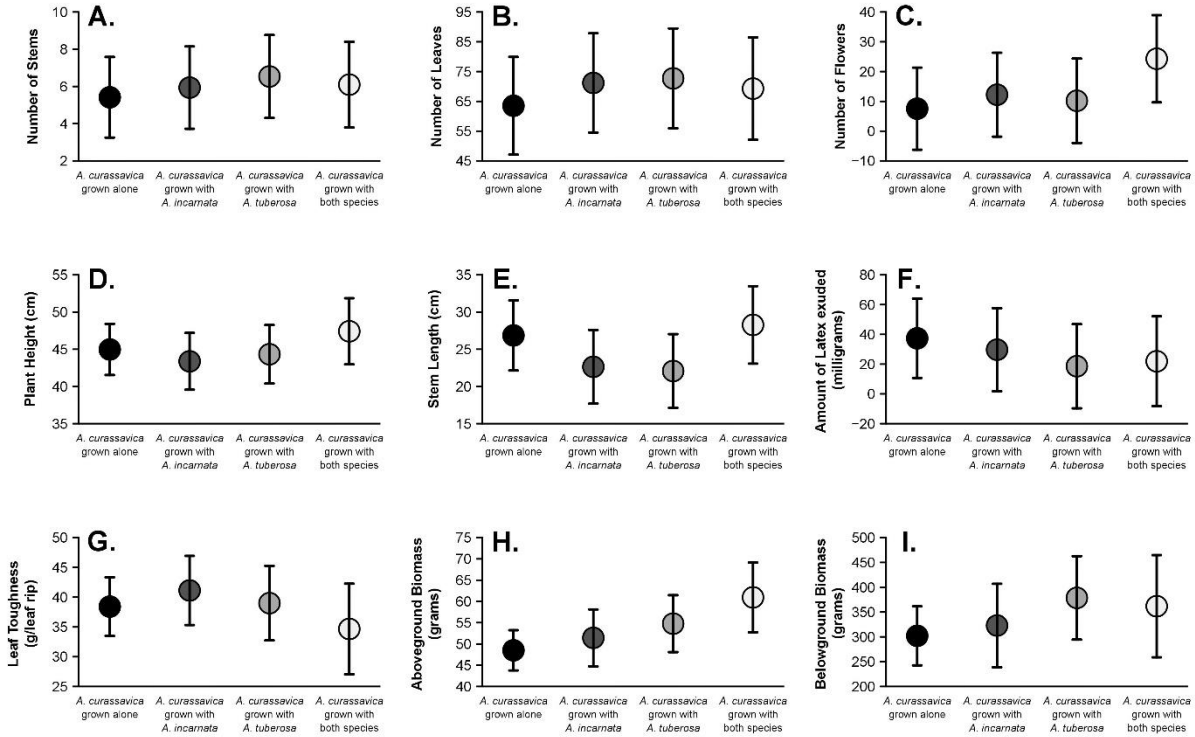

Figure C.7. Fitness metrics of *A. curassavica* grown in competition with native milkweed species across all semesters. Note the commensalistic growth of *A. curassavica* with native milkweed species. (A.) The average number of stems produced by *A. curassavica*, with 95% confidence intervals. *A. curassavica* grown with native species produced the most stems. (B.) The average number of leaves produced by *A. curassavica*, with 95% confidence intervals. (C.) The average number of flowers produced by *A. curassavica*, with 95% confidence intervals. *A. curassavica* grown with both native species produced the most flowers. (D.) The average height of *A. curassavica*, with 95% confidence intervals. (E.) The average stem length of *A. curassavica*, with 95% confidence intervals. *A. curassavica* grown with both species produced the longest stems. (F.) The average amount of latex exuded by *A. curassavica*, with 95% confidence intervals. (G.) The average leaf toughness of *A. curassavica*, with 95% confidence intervals. (H.) The average aboveground biomass of *A. curassavica*, with 95% confidence intervals. (I.) The average belowground biomass of *A. curassavica*, with 95% confidence intervals.

A. INCARNATA FITNESS METRICS

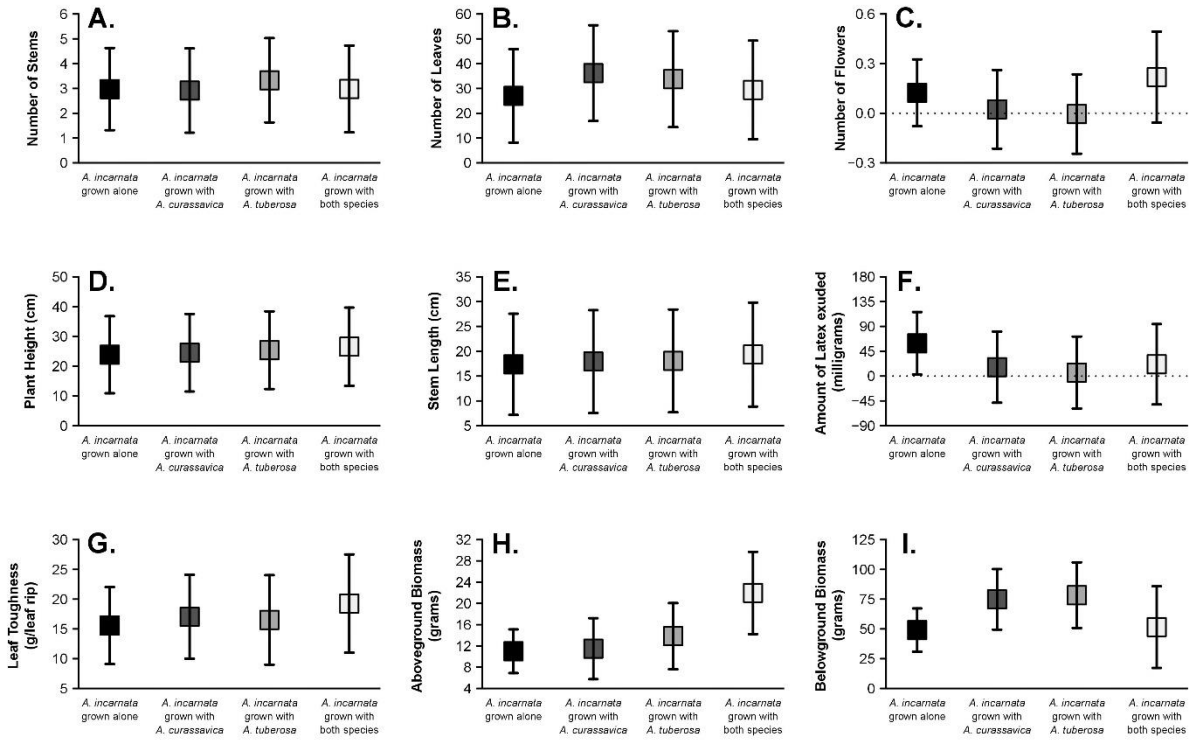

Figure C.8. Fitness metrics of *A. incarnata* grown in competition with both invasive *A. curassavica* and native *A. tuberosa* across all semesters. (A.) The average number of stems produced by *A. incarnata*, with 95% confidence intervals. (B.) The average number of leaves produced by *A. incarnata*, with 95% confidence intervals. (C.) The average number of flowers produced by *A. incarnata*, with 95% confidence intervals. (D.) The average height of *A. incarnata*, with 95% confidence intervals. (E.) The average stem length of *A. incarnata*, with 95% confidence intervals. (F.) The average amount of latex exuded by *A. incarnata*, with 95% confidence intervals. (G.) The average leaf toughness of *A. incarnata*, with 95% confidence intervals. (H.) The average aboveground biomass of *A. incarnata*, with 95% confidence intervals. (I.) The average belowground biomass of *A. incarnata*, with 95% confidence intervals.

A. TUBEROSA FITNESS METRICS

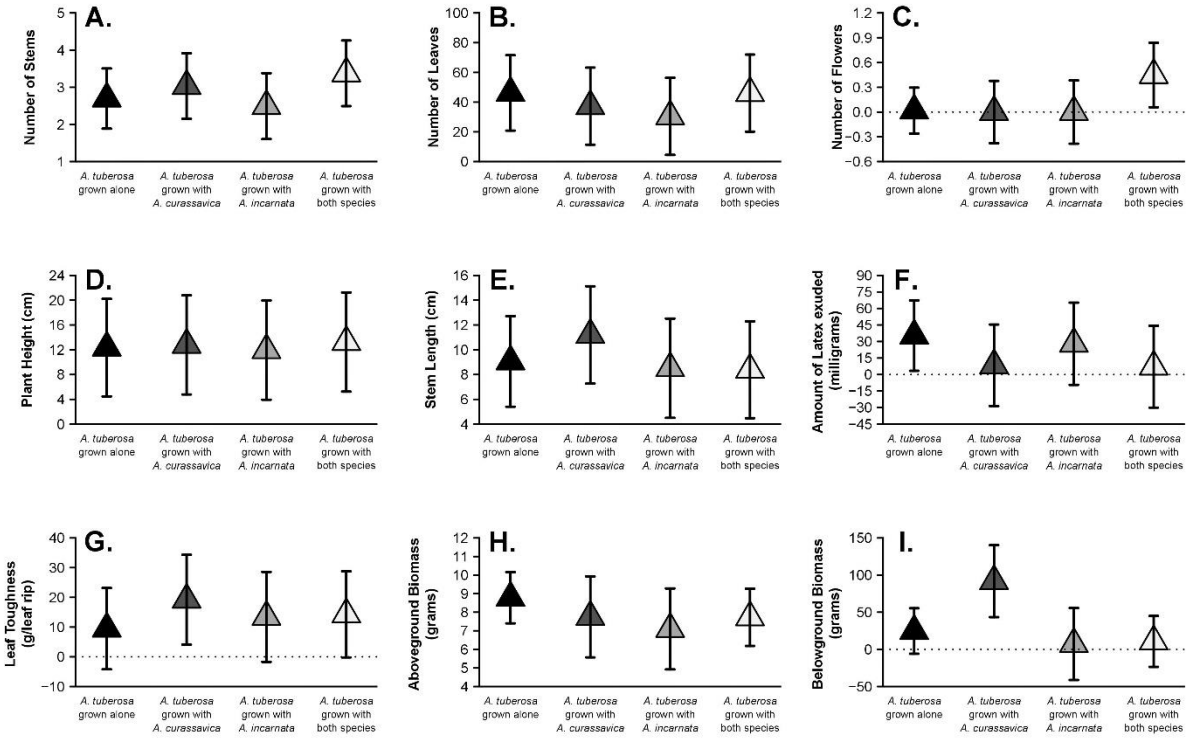

Figure C.9. Fitness metrics of *A. tuberosa* grown in competition with both invasive *A. curassavica* and native *A. incarnata* across all semesters. (A.) The average number of stems produced by *A. tuberosa*, with 95% confidence intervals. (B.) The average number of leaves produced by *A. tuberosa*, with 95% confidence intervals. (C.) The average number of flowers produced by *A. tuberosa*, with 95% confidence intervals. (D.) The average height of *A. tuberosa*, with 95% confidence intervals. (E.) The average stem length of *A. tuberosa*, with 95% confidence intervals. (F.) The average amount of latex exuded by *A. tuberosa*, with 95% confidence intervals. (G.) The average leaf toughness of *A. tuberosa*, with 95% confidence intervals. (H.) The average aboveground biomass of *A. tuberosa*, with 95% confidence intervals. (I.) The average belowground biomass of *A. tuberosa*, with 95% confidence intervals.

GREENHOUSE LAYOUT

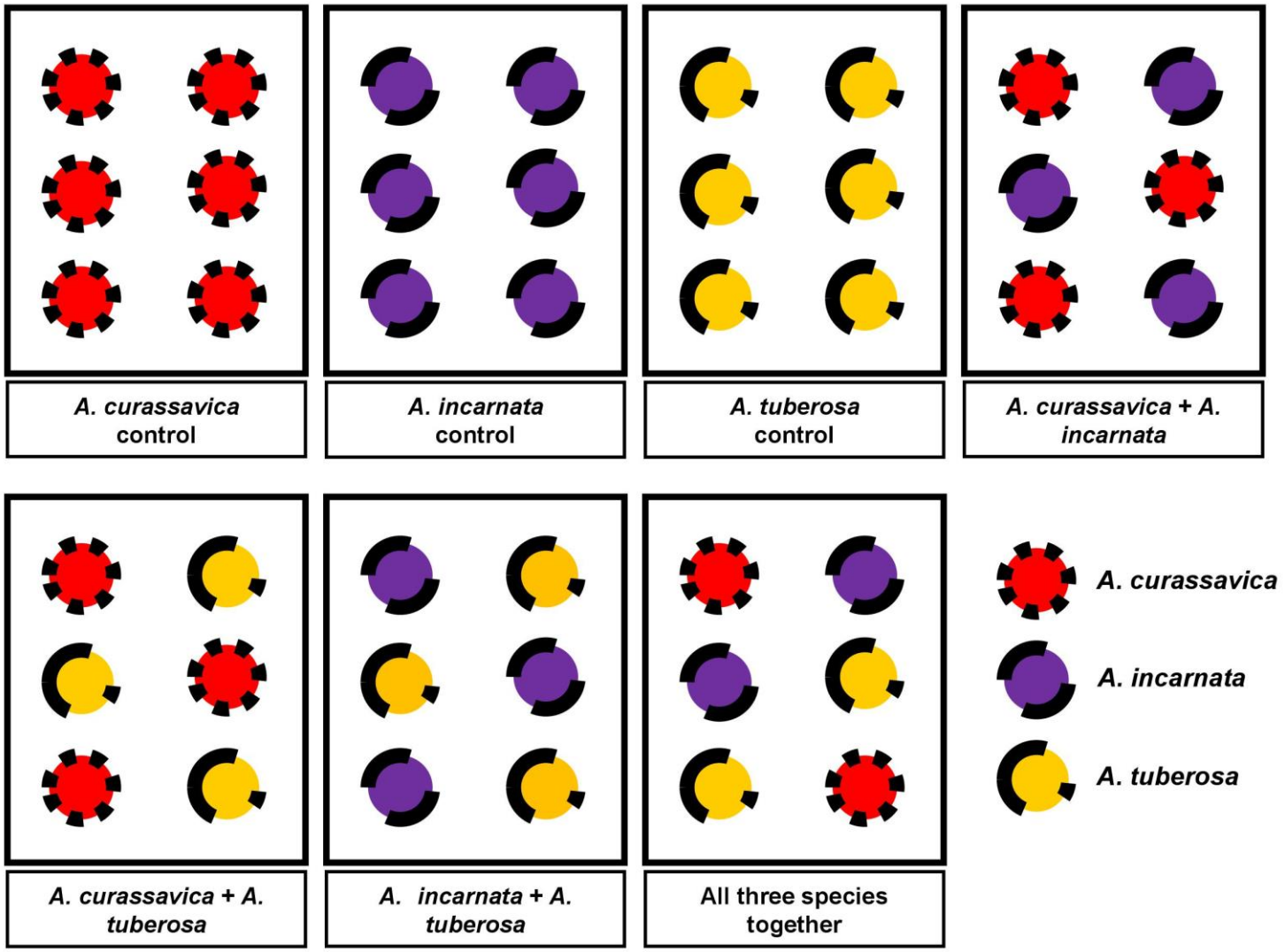

Figure C.10. Layout of the milkweed containers organized in the LSU Greenhouses, AgCenter Horticulture Center, Baton Rouge, LA, USA.

PLANTING ORDER

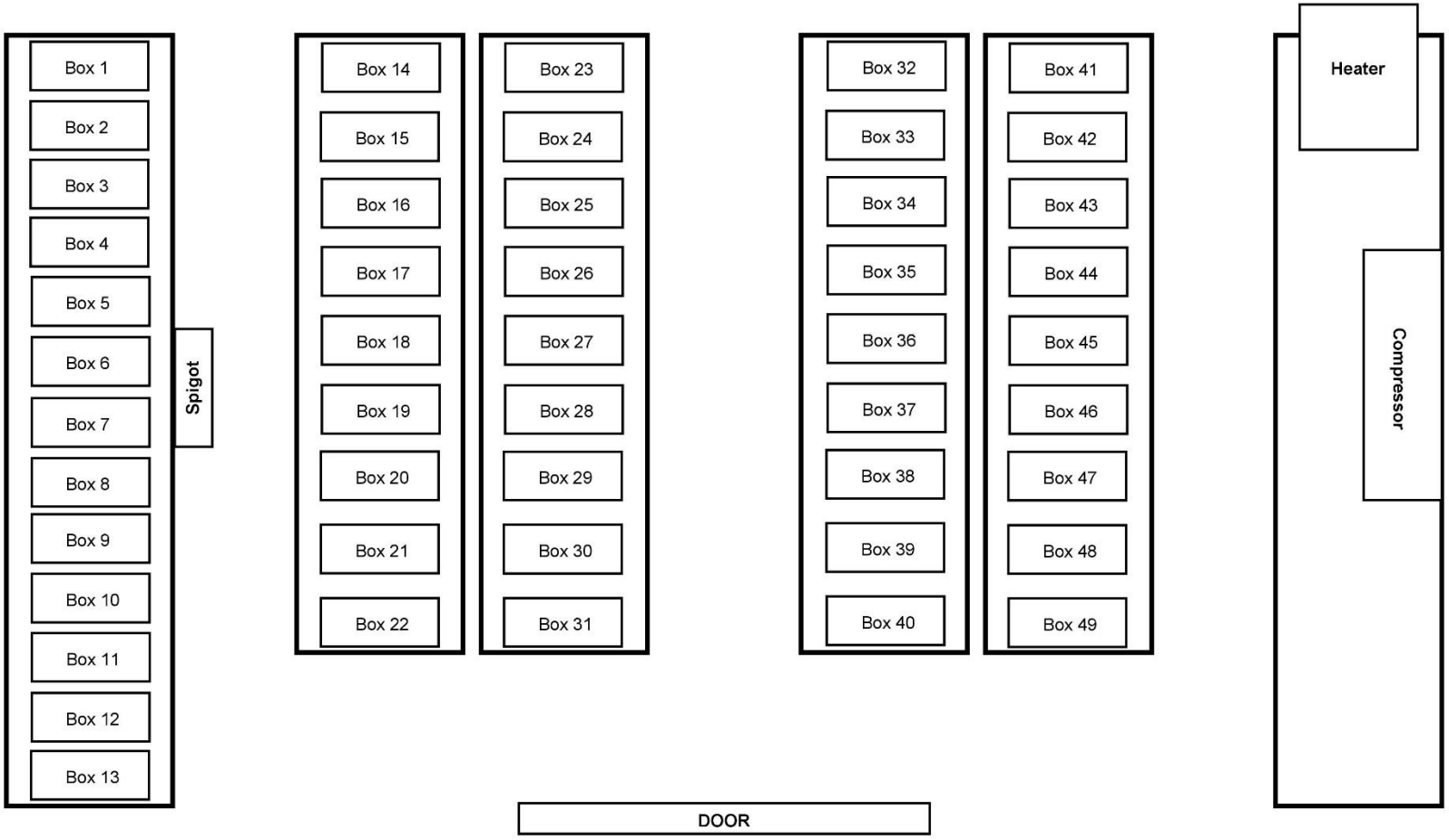

Figure C.11. Planting order for each of the seven treatments used in the competition experiment, with each treatment being replicated seven times per semester.

APPENDIX D. SUPPLEMENTARY CURE COURSE MATERIAL FOR CHAPTER 4

D.1. SURVEY INSTRUMENT

A CURE for Invasive Species - Initial Survey

Survey Flow

Standard: Block 1 (2 Questions)

Branch: New Branch

If

If Would you like to consent and participate in this survey? I consent Is Selected

Standard: Block 2 (12 Questions)
Standard: Block 3 (3 Questions)
Standard: Block 4 (3 Questions)
Standard: Block 5 (10 Questions)
Standard: Block 10 (1 Question)
Standard: Block 11 (3 Questions)
Standard: Block 12 (3 Questions)
Block: Default Block (0 Questions)

EndSurvey:

Page Break

Start of Block: Block 1

Q1
A CURE for Invasive Species: Improving student perceptions of invasive species in Louisiana

Research conducted by:
Matthew J. Faldyn (mfaldy1@lsu.edu)
Dr. William Wischusen
Department of Biological Sciences
Louisiana State University (LSU)

This survey is about “invasive species” in the state of Louisiana. Invasive species are non-native plants and animals that can cause harm to the environment, the economy, and society. You are offered to participate in this survey because you are enrolled in either BIOL 1209 or in BIOL 4253; LSU courses which offer educational insight on invasive species through different classroom methodologies and approaches.

We would like to know about your concerns and beliefs about invasive species and about some of the things you do that could be affected by invasive species. Even if you know very little about invasive species your answers are still very important – you can simply check “Don’t Know” to some of the questions, if needed. The information you provide will help us to improve invasive species education and help protect Louisianans from the negative effects of invasive species in the future.

Your participation in this survey is voluntary, but we sincerely hope you will take just a few minutes to answer these brief questions. The survey should take no more than 10 - 15 minutes to complete. LSU Institutional Review Board (IRB) has verified this survey. Your identity will be kept completely confidential, all names provided will be coded, and all responses will be kept on a secure hard drive.

THANK YOU FOR YOUR HELP!

Q2 Would you like to consent and participate in this survey?

- I consent (1)
- I do not consent (2)

End of Block: Block 1

Start of Block: Block 2

Q44 Course Information

Q3 What is your name?

- First Name (1) _____
- Last Name (2) _____

Q4 Which course are you enrolled in?

- BIOL 1209 (1)
- BIOL 4253 (2)

Display This Question:

If Which course are you enrolled in? = BIOL 1209

Q33 Which section of BIOL 1209 are you enrolled in?

- Section 26 (1)
- Section 27 (2)

Display This Question:

If Which course are you enrolled in? = BIOL 4253

Q34 Which section of BIOL 4253 are you enrolled in?

- Section 1 (1)
- Section 2 (2)

Q35 Regarding Ecology Lab (BIOL 4254), have/are you:

- completed Ecology Lab (Biol 4254) (1)
- currently enrolled in Ecology Lab (BIOL 4254) (2)
- not enrolled and haven't completed/taken Ecology Lab (BIOL 4254) (3)

Q26 Demographic Information

Q27 In what year were you born?

_____

Q28 What is the highest level of education you have completed? (check one)

- Less than high school (1)
- High school diploma / G.E.D. (2)
- Some college or technical school (3)
- Associate's degree (4)
- College undergraduate degree (e.g., B.A., B.S.) (5)
- Graduate or professional degree (e.g., M.S., Ph.D., M.D., J.D.) (6)

Q29 What was the total income of your household before taxes last year? (Check one.)

- Less than $25,000 (1)
- $25,000 to $49,999 (2)
- $50,000 to $74,999 (3)
- $75,000 to $99,999 (4)
- $100,000 or more (5)

Q30 Are you of Hispanic, Latino, or Spanish origin?

- No (1)
- Yes (2)

Q31 What is your race? (Check all that apply.)

- White (1)
- Black or African-American (2)
- Asian or Pacific Islander (3)
- Native American Indian (4)
- Other (5)

End of Block: Block 2

Start of Block: Block 3

Q5 How much would you say you know about invasive species (which are non-native plants and animals that can cause harm to the environment, the economy, and society)?

- Very little (1)
- Something (2)
- A lot (3)

Q6 How concerned are you about having invasive species in Louisiana?

- Not at all concerned (1)
- Slightly concerned (2)
- Moderately concerned (3)
- Very concerned (4)

Q7 Besides invasive species, Louisianans may be concerned about a wide variety of problems and some are more important than others. How important are each of the following problems to you? (Check one box for each statement.)

	Not at all important (1)	Slightly Important (2)	Moderately Important (3)	Very Important (4)
Defending the U.S. against terrorism (1)	○	○	○	○
Dealing with problems of poor (2)	○	○	○	○
Improving the job situation (3)	○	○	○	○
Reducing middle class taxes (4)	○	○	○	○
Strengthening the military (5)	○	○	○	○
Protecting the environment (6)	○	○	○	○
Dealing with global warming (7)	○	○	○	○
Strengthening the nation's economy (8)	○	○	○	○
Reducing budget deficits (9)	○	○	○	○
Reducing healthcare costs (10)	○	○	○	○
Reducing crime (11)	○	○	○	○

End of Block: Block 3

Start of Block: Block 4

Q8 Louisianans have different beliefs about the impacts of invasive species. Before you received this questionnaire, how strongly would you have agreed or disagreed with each of the following statements? (Check one box for each statement.)

	Strongly Agree (1)	Agree (2)	Neutral (3)	Disagree (4)	Strongly Disagree (5)	Do not know (6)
Invasive species can harm wildlife, fish, and ecosystems (1)	○	○	○	○	○	○
Invasive species have negative effects on the economy (2)	○	○	○	○	○	○
Invasive species can harm people's health (3)	○	○	○	○	○	○
Invasive species can interfere with people's ability to make a living (4)	○	○	○	○	○	○
Invasive species can interfere with people's recreational activities (5)	○	○	○	○	○	○
Invasive species can harm domestic animals (6)	○	○	○	○	○	○

Q9 How concerned are you personally about the negative effects of invasive species on each of the following? (Check one box for each statement.)

	Not concerned at all (1)	Slightly Concerned (2)	Moderately Concerned (3)	Very Concerned (4)
Wildlife (1)	○	○	○	○
Fish (2)	○	○	○	○
Ecosystems (3)	○	○	○	○
The economy (4)	○	○	○	○
People's health (5)	○	○	○	○
People's ability to make a living (6)	○	○	○	○
People's recreational activities (7)	○	○	○	○
Domestic animals (8)	○	○	○	○

Q10 How much of a contribution do you believe each of the following activities makes to the spread of invasive species in Louisiana? (Check one box for each statement.)

	No Contribution (1)	Slight Contribution (2)	Moderate Contribution (3)	Large Contribution (4)	Do not know (5)
Recreational boating (1)	○	○	○	○	○
Decorative plantings (2)	○	○	○	○	○
Hiking (3)	○	○	○	○	○
Fishing (4)	○	○	○	○	○
Camping (5)	○	○	○	○	○
Use of ATV's (6)	○	○	○	○	○

End of Block: Block 4

Start of Block: Block 5

Q11 Do you own a boat that you have used in the past year?

○ No (1)

○ Yes (2)

Display This Question:

If Do you own a boat that you have used in the past year? = Yes

Q12 How often do you do each of the following when you use your boat? (Check one box for each statement.)

	Never (1)	Some of the time (2)	Most of the time (3)	Always (4)
Drain all water-holding compartments in your boat when taking it out of a water body. (1)	○	○	○	○
Wash your boat with a hose when you get home. (2)	○	○	○	○
Clean off vegetation that is caught on the boat. (3)	○	○	○	○
Dry boats, trailers and all boating equipment before use in another water body (4)	○	○	○	○

Q37 Have you gone fishing in the past year?

- ○ No (1)
- ○ Yes (2)

Display This Question:

If Have you gone fishing in the past year? = Yes

Q39 How often do you do each of the following when you go fishing? (Check one box for each statement.)

	Never (1)	Some of the time (2)	Most of the time (3)	Always (4)
Buy baitfish that are "certified" disease free. (1)	○	○	○	○
Take leftover bait from one body of water to another. (2)	○	○	○	○
Dump unused bait on dry land or in the trash. (3)	○	○	○	○
Clean your fishing equipment (e.g., rods, reels, and lures) when you are done fishing in a body of water. (4)	○	○	○	○

Q39 Have you gone camping in the past year?

- ○ No (1)
- ○ Yes (2)

Display This Question:

If Have you gone camping in the past year? = Yes

Q41 How often do you do each of the following when you go camping? (Check one box for each statement.)

	Never (1)	Some of the time (2)	Most of the time (3)	Always (4)
Bring firewood with you from home. (1)	○	○	○	○
Take leftover firewood home with you from your campsite. (2)	○	○	○	○
Clean your camping equipment before going home or to a different area. (3)	○	○	○	○

Q43 Have you gone hiking in the past year?

○ No (1)

○ Yes (2)

Display This Question:

If Have you gone hiking in the past year? = Yes

Q45 How often do you do each of the following when you go hiking? (Check one box for each statement.)

	Never (1)	Some of the time (2)	Most of the time (3)	Always (4)
Take plants you find when you are hiking and plant them at home. (1)	○	○	○	○
Clean off your clothes and hiking gear before going home or to a different hiking area. (2)	○	○	○	○

Q47 Have you gardened with flowers and vegetables in the past year?

- ○ No (1)
- ○ Yes (2)

Display This Question:

If Have you gardened with flowers and vegetables in the past year? = Yes

Q49 Which of the following have you done in your garden? (Check all that apply.)

- ○ Removed invasive garden plants (1)
- ○ Replaced invasive garden plants with native or noninvasive plants (2)
- ○ Found out whether a plant was invasive before planting it (3)

End of Block: Block 5

Start of Block: Block 10

Q22 Before you received this questionnaire, how strongly would you have agreed or disagreed with the following statements? (Check one box for each statement.)

	Strongly agree (1)	Agree (2)	Neutral (3)	Disagree (4)	Strongly Disagree (5)	Do not know (6)
Trading or transporting some invasive plants is illegal. (1)	○	○	○	○	○	○
Many fish used in aquariums are not native and may be invasive. (2)	○	○	○	○	○	○
Some common garden and landscaping plants are invasive species. (3)	○	○	○	○	○	○
Invasive species can be transported on trailered boats. (4)	○	○	○	○	○	○
Invasive species can be transported on fishing gear. (5)	○	○	○	○	○	○
Some plants that some people encounter when hiking are invasive species. (6)	○	○	○	○	○	○

There are native substitutes for many invasive garden plants. (7)	○	○	○	○	○	○
Invasive species can be transported in firewood. (8)	○	○	○	○	○	○
Some fish used in aquariums might be able to survive in the wild and invade natural waters. (9)	○	○	○	○	○	○
Some of the things I like to do outside are negatively affected by invasive species. (10)	○	○	○	○	○	○

End of Block: Block 10

Start of Block: Block 11

Q23 If you found out that some of the things you were doing were contributing to the spread of invasive species in Louisiana, how willing would you be to change your behavior?

- ○ Not at all willing (1)
- ○ Possibly willing (2)
- ○ Willing (3)
- ○ Very willing (4)

Q24 If you found out that some of the things you were doing were contributing to the spread of invasive species in Louisiana, how important do you think each of the following reasons would be for changing your behavior? (Check one box for each statement.)

	Not at all important (1)	Slightly important (2)	Moderately important (3)	Very important (4)
Invasive species cost Louisianans money. (1)	○	○	○	○
Invasive species hurt the Louisiana economy. (2)	○	○	○	○
Invasive species interfere with things you like to do. (3)	○	○	○	○
Invasive species interfere with things other Louisianans like to do. (4)	○	○	○	○
Your behavior could be changed without much difficulty. (5)	○	○	○	○
Your behavior could be changed without costing you more. (6)	○	○	○	○
Your behavior could be changed without you having to spend more time. (7)	○	○	○	○

Q25 From which of the following sources (if any) have you gotten information about invasive species? (Check all that apply.)

- ○ TV (1)
- ○ Internet (2)
- ○ Radio (3)
- ○ Newspapers or other print materials (4)
- ○ Friends and family (5)

End of Block: Block 11

Start of Block: Block 12

Q37 Final Thoughts

Q32 Please use the space below for any comments you wish to make.

_____

_____

_____

_____

_____

Q33 Thank you for your time and effort!

End of Block: Block 12

Start of Block: Default Block

D.2. Flight of the Butterflies worksheet

Name: _____ **Date:** _____
Class: BIOL 1209R Spring 2018 **Section #:** _____

ICWA1: Flight of the Butterflies

Directions: Using the topics and ideas presented in the Netflix/National Science Foundation (NSF) documentary titled *Flight of the Butterflies*, complete the following assignment and submit it to me by the end of class for your first grade. Every answer is worth 1pt, unless otherwise denoted.

1. **T or F** Monarch butterflies are an ancient tropical species that have been making their annual migration for thousands of years.

2. List, in order of growth, the four stages of complete metamorphosis of the monarch:

3. Generally speaking, what happens to the monarch and its larval body parts when it enters its chrysalis (pupal) stage? Do any remain the same between larvae and adult? Which ones?

4. **T or F** Less than 1% of monarch eggs reach adulthood.

5. **T or F** The monarch is a highly-evolved migratory insect navigating and orienting itself for a few miles to a remote and small place to which it has never been.

6. **T or F** Monarchs can soar up to a mile high, and they weigh as little as a paper clip.

7. How many generations do monarchs undergo as they make their annual migration? What is the longest living generation called?

8. Monarchs taste with their _____ and smell with their _____.

9. **T or F** Do monarchs lay their eggs on one type of plant? What is that plant called? **Yes or No**; can adult monarchs feed on the plant nectar?

10. **T or F** Various natural predators eat around 90% of the eggs and caterpillars before they form the chrysalis/pupa.

11. The monarch faces a number of human threats. What are two threats posed by human activities, and **in detail**, how does each threat impact monarch butterflies (2pts each)? Use back of sheet to answer. Pictures are allowed if they help make your point.

D.3. Primary literature discussion: Jeschke *et al.* 2014

Discuss the Jeschke 2014 paper in groups of four. Address the following questions during your discussion:

1. What message is the paper attempting to convey?

2. What is the theoretical basis underlying each invasion hypothesis?

3. What do you think of the evidence supporting each hypothesis (strengths and limitations)?

4. What are some criticisms of invasion ecology?

5. What solutions/improvements does the author suggest? Can you think of any others?

6. Did you enjoy the paper? Why or why (not)?

D.4. Take Home Assignment 1

BIOL 1209 R Take Home Writing Assignment 1

Due Jan 30th at 11:59 pm (upload to Moodle unless told otherwise)

Jeschke JM (2014) General hypotheses in invasion ecology. Diversity and Distributions 20:1229-1234.

Assignment:

- Provide a 2 page summary/critique of one of the four hypotheses covered by Jeschke (2014) or an additional different hypothesis you are interested in researching (let me know first if you want to do a different one).
- Use at least 3 additional references not included in the original paper to back up your argument for or against the hypothesis (use Google scholar, Web of Knowledge)
- Provide references section at end of paper (not included in page limit)
- **Rules: Double spaced, 1in margins, 12pt font, times new roman or arial font, normal letter and line spacing**
- Follow standard scientific writing protocols (italicize species names, use standard units, proper grammar and consistency of formatting, etc) – see a journal such as *Ecology* for examples

C**onsider the following questions/ideas to help guide your writing:**

- What is the premise and history behind the hypothesis you have chosen to write about?
- What are some ways in which the hypothesis has been tested?
- What limitations are there to testing the hypothesis?
- What evidence is there in support of the hypothesis, and is the evidence of good quality?
- What evidence is there against the hypothesis, and what is its quality?
- What future research directions could help us further understand the hypothesis?
- What is your conclusion regarding the hypothesis

Useful writing tips:

- Begin with broad outline of topic and then narrow in focus (tree roots).
- Organize your ideas into paragraphs – start each paragraph with a topic sentence.
- Be concise
- Revise, revise, revise!
- Read what you have written aloud to yourself.
- Use spellcheck.
- Have someone else proofread your writing.
- Look at the style of writing style of Jeschke (2014), Mack et al. (2000) and other suggested readings – try to emulate this scientific style of writing in your own assignments this semester.

D.5. Take Home Assignment 2

BIOL 1209R Take Home Writing Assignment 2

Based on your own specific experiment, write an introduction, methods, and references sections suitable for a scientific paper. This writing assignment should be your INDIVIDUAL WORK. Please refer to the grading rubric on Moodle. Specific information for each required section is given below:
SEE TIPS AND TRICKS DOCUMENT ON MOODLE

Title

- Descriptive and concise
- See scientific papers for examples

Introduction

- Background information and biological relevance: put your research into context
- Clearly state your research question
- Provide a reference when making statements of scientific fact with studies to support them
- *Look at scientific papers as examples* (I cannot stress this enough)

✓ Paragraphs must be cohesive with a nice flow and good transition statements. The paragraph should be developed as a "funnel" – where you go from making broad general statements regarding ecological theory to getting more specific about your particular experiment
✓ Must be concise – no more than 1 page (single spaced)

Methods

- Summarize the major procedural steps for the experiment performed
- Include all detail necessary for someone to repeat the experiment without having to contact you
    - Experimental design and set-up (details are important – how much soil, fertilizer, pot size, watering regime, etc.)
    - Important dates (planting date, data collection, how many times will we collect? - check schedule!)
    - Species used (with scientific names)
    - Data collection methods
    - Data analyses used (use SAS-JMP program)
    - Labeled diagrams of methodology or photographs of experimental set-up (if appropriate)

✓ You need to include all relevant & important information here but not trivial things. See examples below:
    - *Example 1:* Do not say how many pots were planted, but say how many replicates there are of each treatment

    - *Example 2:* You need to say what size pots were used (bussing tubs), soil type (swamp soil), how data was collected, but not that plants were labelled using wooden popsicle sticks and sharpies
- ✓ DO NOT write this in a step-by-step format, it needs to be in paragraph form
- ✓ DO NOT include any results here! Only describe what you did, not what you found
- ✓ Methods section should be written in past tense

References

- Must include at least three references in introduction/reference list
- Journal articles and books only
- Use correct formatting styles (see below or powerpoint slides for examples)
- Correct formatting in text:
    - ✓ (Smith 2005) – one author
    - ✓ (Smith and Wesson 2006) – two authors
    - ✓ (Smith et al. 2007) – three or more authors
- Journal article citation formatting:
    - ✓ Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.
- Book citation formatting:
    - ✓ Elton CS (1958) The ecology of invasions by animals and plants. University of Chicago Press, Chicago, IL. 196p.

Useful writing tips:

- Begin with broad outline of topic and then narrow in focus (tree roots).
- Organize your ideas into paragraphs – start each paragraph with a topic sentence.
- Be concise
- Revise, revise, revise!
- Read what you have written aloud to yourself.
- Use spellcheck.
- Have someone else proofread your writing.
- Look at the style of writing style of Jeschke (2014), Mack et al. (2000) and other suggested readings – try to emulate this scientific style of writing in your own assignments this semester.

D.6. Formal Writing Assignment 1

BIOL 1209 R Formal Writing Assignment 1

Based on your own specific experiment, write an introduction, methods, and references sections suitable for a scientific paper. This writing assignment should be your INDIVIDUAL WORK. Please refer to the grading rubric on Moodle. Specific information for each required section is given below:

Title (10 points)

- Descriptive and concise
- See scientific papers for examples

Introduction (50 points)

- Background information and biological relevance: put your research into context
- Clearly state your research question
- Provide a reference when making statements of scientific fact with studies to support them
- *Look at scientific papers as examples* (I cannot stress this enough)
- ✓ Paragraphs must be cohesive with a nice flow and good transition statements. The paragraph should be developed as a "funnel" – where you go from making broad general statements regarding ecological theory to getting more specific about your particular experiment
- ✓ Must be concise – no more than 1 page (single spaced)

Grade Breakdown for this section:

- ➢ Background/relevance/context: 20 points
- ➢ Research question: 10 points
- ➢ Sources properly referenced: 10 points
- ➢ Style and flow: 10 points

Methods (30 points)

- Summarize the major procedural steps for the experiment performed
- Include all detail necessary for someone to repeat the experiment without having to contact you
    - Experimental design and set-up (details are important – how much soil inoculum, fertilizer, pot size, watering regime, etc.)
    - Important dates (planting date, data collection, how many times will we collect? - check schedule!)
    - Species used (with scientific names)
    - Data collection methods
    - Data analyses used (use SAS-JMP program)
    - Labeled diagrams of methodology or photographs of experimental set-up (if appropriate)

- ✓ You need to include all relevant & important information here but not trivial things. See examples below:
    - *Example 1:* Do not say how many pots were planted, but say how many replicates there are of each treatment
    - *Example 2:* You need to say what size pots were used (bussing tubs), soil type (swamp soil), how data was collected, but not that plants were labelled using wooden popsicle sticks and sharpies
- ✓ DO NOT write this in a step-by-step format, it needs to be in paragraph form
- ✓ DO NOT include any results here! Only describe what you did, not what you found
- ✓ Methods section should be written in past tense
- ✓ Proper flow and logic + no more than 1 page (single spaced)
- ✓ Be concise

Grade Breakdown for this section:

- ➢ Techniques properly & concisely described as instructed: 25 points
- ➢ Style and flow: 5 points

References (10 points)

- Must include at least three references in introduction/reference list
- Journal articles and books only
- Use correct formatting styles (see below or powerpoint slides for examples)
- Correct formatting in text:
    - ✓ (Smith 2005) – one author
    - ✓ (Smith and Wesson 2006) – two authors
    - ✓ (Smith et al. 2007) – three or more authors
- Journal article citation formatting:
    - ✓ Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.
- Book citation formatting:
    - ✓ Elton CS (1958) The ecology of invasions by animals and plants. University of Chicago Press, Chicago, IL. 196p.

Useful writing tips:

- Begin with broad outline of topic and then narrow in focus (tree roots).
- Organize your ideas into paragraphs – start each paragraph with a topic sentence.
- Be concise
- Revise, revise, revise!
- Read what you have written aloud to yourself.
- Use spellcheck.
- Have someone else proofread your writing.
- Look at the style of writing style of Jeschke (2014), Mack et al. (2000) and other suggested readings – try to emulate this scientific style of writing in your own assignments this semester.

D.7. Formal Writing Assignment 2

BIOL 1209 R Formal Writing Assignment II

Based on your groups specific experiment, write a results, discussion, and references sections suitable for a scientific paper. Only submit one paper per group for grading. This writing assignment should be your based on your OWN group's work. Please refer to the grading rubric on Moodle. Specific information for each required section is given below:

Title

- Descriptive and concise
- See scientific papers for examples
- Example:
    - "Understanding how invasive tropical milkweed (*Asclepias curassavica*) negatively impacts native milkweed species, swamp milkweed (*Asclepias incarnata*) and butterfly weed (*Asclepias tuberosa)"*

Results:

For each independent variable:

- Did the analysis detect significant results? – report all relevant stats (df, F or t stat, p-value, R2) If significant, interpret the direction (for ANOVA – which group was larger, for regression, interpret the slope)
- You should only report the results sentences with the stats, save the description for what the results mean for your discussion

Figures:

- Axis labels with units
- Figure caption – descriptive and in the right location
- Be sure to include equation if a regression is involved, error bars for histogram.

Discussion:

- Start with your study and then broaden the discussion out to the big picture
- Do not simply use the discussion section from the stats lab as your discussion and don't repeat p-values, etc. in the discussion.
- How do the regions differ in the variables you chose to measure? Provide a possible explanation for why they might differ and include references. Is your hypothesis supported or rejected? Deviations from what you expected or predicted – why do you think your results deviated? Do some reading, don't just discredit yourself.
- Paragraphs must be cohesive with a nice flow and good transition statements. The paragraph should be developed as a "reverse funnel" – where you go from making statements regarding what your results mean about your particular experiment to how they relate to invasions broadly
- See posters for examples
- Must be concise – no more than 1 page (single spaced)
- Derive conclusions and about what drives those differences (use the literature to relate your findings to other similar studies) Relate your findings to other relevant studies.

- Repeat for other variables you may have examined.
- Future directions and ways to improve the experiment?

Literature Cited:

- Should be listed alphabetically by 1st author
- Must have 3 primary sources – textbooks, internet sources (including Wikipedia) don't count
- Journal articles and books only
- Use correct formatting styles (see below or PowerPoint slides for examples)
- Correct formatting in text:
  - ✓ (Smith 2005) – one author
  - ✓ (Smith and Wesson 2006) – two authors
  - ✓ (Smith et al. 2007) – three or more authors
- Journal article citation formatting:
  - ✓ Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.
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  - ✓ Elton CS (1958) The ecology of invasions by animals and plants. University of Chicago Press, Chicago, IL. 196p.

Useful writing tips:

- Begin with broad outline of topic and then narrow in focus (tree roots).
- Organize your ideas into paragraphs – start each paragraph with a topic sentence.
- Be concise
- Revise, revise, revise!
- Read what you have written aloud to yourself.
- Use spellcheck.
- Have someone else proofread your writing.
- Look at the style of writing style of Jeschke 2014, Mack *et al.* 2000 and other suggested readings – try to emulate this scientific style of writing in your own assignments.

D.8. Frequentist statistics assignment (badger dataset)

Badger Stats Homework: JMP Computer Assignment

For your statistics homework assignment, you will need to complete the data analysis for the data given below. Please complete pages 2 and 3 on one Word Document and submit that to Moodle.

Table 1. Home range (100 m^2) was determined for nine male badgers, and compared against prey density (rodents per 100 m^2) and predator density (wolverines per 100 m^2).

Home range	Prey density	Predator density
10	1.2	0.5
5	1.5	1.0
16	1.6	0.3
4	1.3	0.7
13	1.1	0.5
18	1.5	0.4
2	1.3	0.8
14	1.2	0.4
9	0.8	0.4

Table 2. Five individuals from each demographic class were monitored, and home range was measured.

Range (100 m^2)	Demographic
1.2	males
1.0	males
1.6	males
0.8	males
1.4	males
0.9	females
0.6	females
0.6	females
0.3	females
0.7	females
1.6	young
1.5	young
1.0	young
1.2	young
1.2	young

Name:
Section Number:
Pattern A: Home range of badgers varies within the plains.
Hypothesis $_1$: Food supply affects badger home range size.

Prediction 1: As rodent density decreases, male badger home range will increase.
Reasoning: Rodents are a primary food source for badgers, and when rare, badgers may have to forage more widely.

Analytical Approach:

Independent Variable:
Dependent Variable:

Hypothesis $_2$: Predator density affects badger home range size.

Prediction 2: As wolverine density increases, male badger home range will decrease.
Reasoning: It's dangerous to move widely when predators are common.

Analytical Approach:

Independent Variable:
Dependent Variable:

Hypothesis $_3$: The home range size of badgers is differing among badger demographics.

Prediction 3: Male badgers will have the largest home range size.

Reasoning: Males are more dominant and defend larger territories.

Analytical Approach:

Independent Variable:
Dependent Variable:

Results

Regression

- Insert the two scatterplots on badger home range size that you constructed.
- Interpret your results and report the statistics from prey density using the following as an example.
  - Prey density is not significantly correlated to the home range size of badgers ($F_{1,7}$=0.224, p=0.65, R^2=0.031, Figure 1).
- Interpret your results and report statistics from predator density following the example above.
- Interpret the slope only if the relationship is significant. As predator density increases by _____, the home range size increases/decreases by_____ 100 m^2.

ANOVA

- Insert bar graph of demographic classes and home range size.
- Interpret your results and report the statistics from demographic classes using the following example.
  - Home range size differs among the demographics of badgers ($F_{2,12}$ = 9.76, p=0.003, Figure 3). Males have a significantly larger home range than females (p=0.012).
- Add the results of the other two *post hoc* Tukey tests.

Discussion – to be written in paragraphs, not bullets.

Paragraph 1

- Did you reject/fail to reject the hypothesis that the home range of male badgers is not affected by food supply?
- Do your results support the prediction that as rodent density decreases, male badger home range will increase?
- Does food supply cause badgers to have variable home range sizes?

Paragraph 2

- Did you reject/fail to reject the hypothesis that home range of badgers is not affected by predators?
- Do your results support the prediction that as wolverine density increases, male badger home range will decrease?
- Does the threat of wolverine predation pressure cause badgers to have variable home range sizes? Is the relationship positive or negative?
- Which is more important in determining badger home range size – food supply or predation pressure?

Paragraph 3

- Did you reject/fail to reject the hypothesis that the home range size of badgers is not different among badger demographics?
- Which badger demographic has the smallest home range size? Largest? Be careful about interpreting non-significant results.
- Do your results support the prediction that male badgers will have the largest home range size?

.

D.9. Peer review assignment

Peer Review Instructions *(adapted from R. Burner)* **– please read carefully**
Each student will be given another student's Intro and Methods rough draft to evaluate. This will improve the final product and help make the reviewers better writers as well. You can either print this page out along with the paper you're reviewing, fill it in, and scan it. OR, you may fill this out electronically by copying/pasting this paper to the TOP of the paper you're reviewing. To complete this assignment, do the following:

1) **READ the entire paper once through to get an overall feel for what was covered and the writing style**
2) **Go through the checklist item by item. For each item, you need to:**
    a. **UNDERLINE or COPY the section(s) in the paper that fulfill each requirement.**
    b. **LABEL the underlined section(s) with the checklist item NUMBER.**
    c. **WRITE 'YES' or 'NO' in the blank next to each item of the checklist**
3) **RATE the section according to the criterion listed below the checklists (1 to 5 [1 = best]).**
4) **Provide COMMENTS and suggestions for improvement in the comments lines below each section**
5) **CIRCLE any sentences, phrases, or words that you don't understand or that are unclear**
6) **Submit this document and the edited copy of the paper on Moodle**

TITLE checklist
_____Concise
_____Gives overview of research topic and experiment

INTRODUCTION checklist
_____Sections about invasions/theory written in *present tense* (e.g. Invasion ecology is….)
_____Sections about our research written in *past tense* (e.g. We hypothesized that…..)
_____Defines *invasions* for the reader, and discusses what *makes a species invasive*
_____Background information on *invasive species problem* and *invasion ecology*
_____Tells why invasions are a *problem*
_____Background information on *A. curassavica*, its *effects*, and problems in *Louisiana*
_____Mentions *theory(-ies) of invasion*, and says we studied *competition*
_____Provides *citations* for all of the above
_____Discusses *A. incarnata* and *A. tuberosa* and includes common names
_____States *research question* clearly
_____States *hypothesis* clearly
_____States specific *prediction(s)* clearly

Introduction Ratings:

Grammar	1 (great)	2	3	4	5 (needs work)
Logical flow/style	1 (great)	2	3	4	5 (needs work)
Citation formatting	1 (great)	2	3	4	5 (needs work)
Overall completeness	1 (great)	2	3	4	5 (needs work)

Introduction Comments:

METHODS checklist

_____Written in *past tense*
_____Includes *'I'* and *'we'* and mostly *avoids passive voice* (e.g. plants were planted…)
_____Starts with a 1-3 sentence *overview* of our experiment (plants, greenhouse, growth)
_____Describes where we got the *plants* and did the *experiment*
_____Lists all *7 treatments* AND *why* we chose them
_____Mentions the *controls* in our experiment
_____Describes mechanics of the setup (soil, bins, drain holes)
_____Describes watering regime (schedule)
_____Describes the *layout* of the experiment, distribution of treatments
_____Figure describing layout, if present, is clear, referred to in the text, and has a descriptive caption that is located below it
_____Describes data collected, including description of each measurement taken
_____Describes the *schedule* of the experiment (includes *dates* of planting and data collection)
_____Covers material presented on Moodle

Methods Ratings:

Grammar	1 (great)	2	3	4	5 (needs work)
Logical flow/style	1 (great)	2	3	4	5 (needs work)
Overall completeness	1 (great)	2	3	4	5 (needs work)

Methods Comments:

WHAT DID THE AUTHOR OF THIS PAPER DO THE **BEST**?

_____

WHAT 3 THINGS WOULD MOST **IMPROVE** THIS PAPER?

1._____
2._____
3._____

D.10. Quiz 1

Name: _____ **Section #:** _____

Quiz 1

1. Four out of the six steps in the scientific method are listed below in sequential order. Fill in the two missing steps (2 points).

- Pattern identification (observation)
-
- Create predictions
- Hypothesis testing (conduct experiment)
- Interpret results
-

2. The work you are performing in this CURE lab will better inform local plant nurseries, concerned citizens, and scientists about how milkweed communities are changing. What insect species will only lay its eggs on milkweed (*Asclepias* sp.) plants? It was the focal species of the Documentary we watched. Give me the common and scientific name. (2 points).

Common name:

Scientific name:

3. Name <u>four</u> different methods which can be used to communicate the results of science (2 points)

-
-
-
-

4. Complete the following sentence: invasive species are exotic species which cause environmental harm, _____ harm, or harm to human health (1 point).

5. Give the names of <u>two</u> species which are invasive in Louisiana (1 point).

-
-

6. List <u>two</u> traits which are likely to help make an exotic species invasive (2 point).

-
-

D.11. Quiz 2

Name: _____ **Section #:** _____

Quiz 2

Scenario: In the 1960s, the pesticide DDT was commonly used to kill disease spreading insects. DDT accumulates in the bodies of animals high up on the food chain, and causes many negative effects. It's believed that for birds, DDT accumulation in adults interferes with calcium production for egg shells, leading to eggs with shells too thin to support embryos. Thus, many species of large birds (bald eagles, etc.) will produce eggs that are not viable, and those populations will decline. DDT was banned for use in the USA in 1972.

You, a researcher, want to connect the dots between DDT and egg survival rates. You suspect that prior to the 1972 DDT ban, rates of egg survival decreased, and after the DDT ban rates of egg survival increased. In trying to design an experiment:

1. What would be an appropriate research hypothesis? (1pt)

2. What would be the appropriate statistical hypotheses? (2pt)

Null:

Alternative:

3. If you were to perform this experiment, what kind of data would you be collecting? (1pt)

4. Briefly explain TWO of the mandatory requirements for genetic equilibrium. (2 points).

5. The evolutionary development of _____ tissue allowed plants to grow to such large sizes. (i.e. what tissue allows Redwoods to exist?) (1 point)

6. Draw lines to match the plant divisions/groups below to their common names (2 points).

Pterophyta	Horsetails
Anthophyta	Angiosperms
Sphenophyta	Club mosses
Lycophyta	Ferns

5. Correctly spell the scientific name for ONE additional plant division NOT already listed above (1 points).

D.12. Quiz 3

Name: _____ **Section #:** _____

Quiz 3

1. A scientist is interested in whether the success of the invasive wetland plant water hyacinth (*Eichhornia crassipes*) in North America, Asia, and Africa is due to escape from its natural enemies (herbivores) in its native range, the Amazon (e.g., the enemy-release hypothesis). Water hyacinth has an enemy in it's native range, the hyacinth weevil.

Outline a study design which may be used to successfully test the enemy-release hypothesis in the above scenario. Tell me the sampling locations (where would you do it?), an experimental design (draw or write it out- like our greenhouse layout), the treatments you would use (minimum 2), and the types of data which could be collected and how they would be collected (5 points).

2. In the example from question 1, what is your independent and dependent variable? (2 points)

Independent:

_____

Dependent:

_____

3. Write one of the research hypotheses (prediction + reasoning) and a thorough prediction for this Sections milkweed experiment (1 pts):

Hypothesis:

_____

_____

Prediction:

_____

_____

4. Name a native plant species we are using in the competition experiments (1 points (common name) + 1 bonus point if you can give a correctly-spelled scientific name).

Common: _____ Scientific: _____

5. True or False: Randomization and replication are used in experimental studies to account for error associated with variables which are unmeasured or unaccounted for (1 point).

D.13. Quiz 4

Name: _____ **Section #:** _____

Quiz 4

1. Why do we need statistics? Explain your answer in terms of populations vs. samples, comparing means, etc. (2 points):

2. Calculate the mean of the following data (show work): 2, 4, 8, 3, 3, 4 (2 points).

$$\text{mean } (\bar{x}) = \frac{\sum x_i}{n}$$

3. What is the standard deviation of the following data (show work): 2, 2, 2, 2, 2, 2 (1 point)?

$$\text{standard deviation } (s) = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)}}$$

4. Based on your knowledge of native v. invasive species competition, research predictions, and graphing skills, complete the graph below to show the expected results if invasive *A. curassavica* growth *negatively* impacts the growth of *A. incarnata*. *Remember to include 95% CI error bars and give your figure an appropriate caption* (4 points).

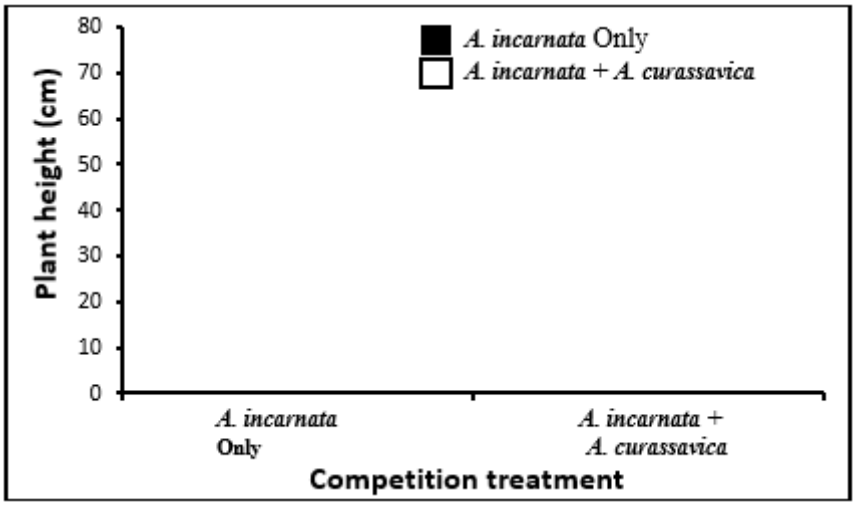


Figure 1.

5. Measuring plant height may not be the most accurate method to estimate plant performance. With this in mind, suggest ONE sensible alternative for data we may gather to estimate plant performance (i.e., what other metrics did we collect?) (1 point).

D.14. Quiz 5

Name: _____ **Section #:** _____

Quiz 5

Insects often develop eyespots, or "fake eyes", to ward off and scare away predators. After noticing caterpillars around LSU's campus having these eyespots, you ask yourself "how effective are eyespots in warding off predators?". You decide to test this, so you design two experiments.

Exp. 1) You plan to make clay caterpillars with no eyespots, some with 1 eyespot, and some with 2 eyespots. You plan to make 30 of each caterpillar type, and you randomly place them all around plants on campus. After some time, you collect the clay caterpillars and check how many have been attacked. You predict that caterpillars with more eyespots will experience less predation (less attacks) on average.

Exp. 2) Additionally, you think that the longer you leave the caterpillars outside, the more they will be attacked. So, you stagger the collection time of the caterpillars where you collect 30 of the caterpillars per day, at random times, over a three-day period, and record how long the caterpillar was left outside along for with the number of attacks. You predict caterpillars left outside longer will experience more predation (more attacks).

1) **What would be the null hypothesis for Exp. 1? (1pt) (hint: think about the data you collected)**

2) **What would be the null hypothesis for Exp. 2? (1pt) (hint: think about the data you collected)**

3) **What kind of statistical test would you use to analyze the data from Exp. 1 and Exp. 2? (3pt)**

Exp. 1

_____

Exp. 2

_____

4) **Using your knowledge of presenting results and making figures, draw a hypothetical graph of the results for Exp. 1 and for Exp. 2. <u>There are significant differences and relationships</u>. Include figure captions, axes labels, units, etc.! Remember, error bars (when they're drawn) are (+/- 95%CI) (8pts total, 4pts per figure)**

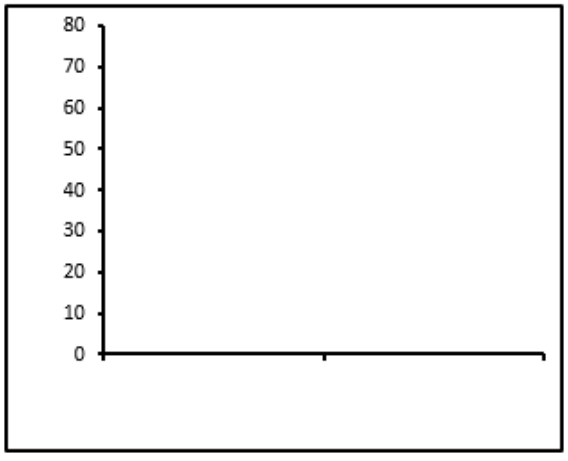


Figure 1)

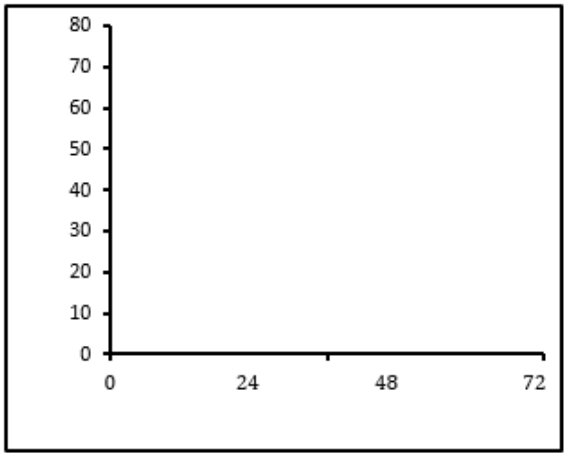


Figure 2)

D.15. Quiz 6

Name: _____ **Section #:** _____

Quiz 6

Using the poster image posted below, list three things wrong with the poster. For each critique point, include what you would recommend be done to remedy the issue- be specific and descriptive!

a. Critique #1 (1pt):

_____

_____

Recommendation (1.5pts):

_____

_____

b. Critique #2 (1pt):

_____

_____

Recommendation (1.5pts):

_____

_____

c. Critique #3 (1pt):

_____

_____

d. Recommendation (1.5pts):

_____

_____

2. To help Dr. Paige Jarreau write a blog post about the project, what are your thoughts about the class and research? What did you enjoy most? What did you learn? What's been the most challenging? What was your most surprising finding? (2.5 pts)

_____

_____

_____

_____

_____

_____

_____

_____

D.16. Quiz 7 (bonus)

Name: _____ **Section #:** _____

Quiz 7 (BONUS)

In the 1920s, gray wolves were hunted and killed by farmers and government officials as a means of predator control in and around Yellowstone National Park. You are the chief park ranger in Yellowstone, and you think that with gray wolves removed, moose in the park may no longer have any natural predators. You suspect that without predators in the park, the moose population in Yellowstone will rapidly increase, explaining why many trees, grasses, and shrubs throughout the park were now stripped of vegetation. Tourists were visiting the park much less often, and the U.S. National Parks service has tasked you with figuring out what's going on. You decide to run an experiment to see how the presence of wolves affects Yellowstone National Park. You quantify the amount of aboveground biomass remaining (tons/Ha^2) at certain sites, along with the number of wolves present in those areas. Next, you and your team build fences around areas with no wolves, 2 wolves present, and 4 wolves present and quantify the amount of aboveground biomass remaining (tons/Ha^2) at those sites. You collect the data, run the statistics, and now have to address the Board of Officers.

Regression

Summary of Fit

RSquare	0.790857
RSquare Adj	0.783388
Root Mean Square Error	101.9701
Mean of Response	501.9667
Observations (or Sum Wgts)	30

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1100927.9	1100928	105.8799
Error	28	291141.1	10398	Prob > F
C. Total	29	1392069.0		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	162.92589	37.84505	4.31	0.0002*
Number of Wolves Present	44.610629	4.335425	10.29	<.0001*

ANOVA/Tukey HSD tests

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Yellowstone treatment	2	1277469.3	638735	150.4876	<.0001*
Error	27	114599.7	4244		
C. Total	29	1392069.0			

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
4 Wolves Present	No Wolves Present	505.3000	29.13566	433.0605	577.5395	<.0001*
2 Wolves Present	No Wolves Present	263.8000	29.13566	191.5605	336.0395	<.0001*
4 Wolves Present	2 Wolves Present	241.5000	29.13566	169.2605	313.7395	<.0001*

5) What would be the results from the regression? (2pts) (include relevant statistics!)

6) Results from the ANOVA (3pts) (include ALL relevant statistics!)

7) Using your knowledge of presenting results and making figures, draw a hypothetical graph of the results for the experiments above. <u>Are there are significant differences and relationships</u>? Include figure captions, axes labels, units, etc.! Remember, error bars (when they're drawn) are (+/- 95%CI) (5pts total, 2.5pts per figure

Figure 1)

Figure 2)

D.17. Student sample 1: Lightning talk

Biol Invasions (2012) 14:1237–1250
DOI 10.1007/s10530-011-0152-2

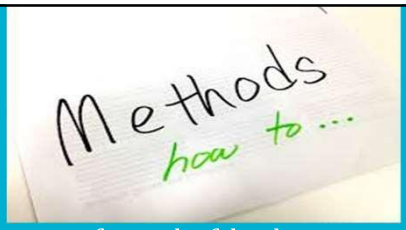
ORIGINAL PAPER

Modes of reproduction in three invasive milkweeds are consistent with Baker's Rule

Megan Ward · Steven D. Johnson · Myron P. Zalucki

Presented by: Parker Hannifan, Megan Mahoney

Method



The milkweed experiment was broken up into three separate treatments for each of the three types of

- Hand-pollination was used
- 3 umbels ("50 plants per population")
- Plants were covered in mesh cloth to prevent others from pollinating the plants treatment to test for autonomous self-pollination
- The umbels were checked daily
- After 5-8 weeks the plants were checked for fruit/ if they had died
- "The number of filled seeds per fruit was counted"

Methods Continued

A hybridization experiment was also done. This was done to study if hybridization occurred between the

- Two umbels (25 more plants) were
- obtained Hand-pollination was done
- "Each umbel received pollinia from one of the two other species"
- "Three flowers per umbel each received a single pollinium"
- The flowers were marked individually with a colored string according to the
- treatment Bags were placed over the plants to prevent other pollinators

Methods

A performance of progeny experiment was also done. This was done to "compare the performance of selfed

- 300 seeds per species per treatment were used; 30 seeds randomly selected from ten plant
s 100 seeds per treatment were for hybridization
- Seeds were moistened the sealed with Parafilm (to minimize desiccation)

temperature of 23 degrees

- After two leaves had grown, 20 seedlings from each of the crossed and selfed fruit randomly selected and were plotted in general-purpose potting mix.
- The potted plants were randomly placed
- Plant height was measured after 8 weeks for the A curassavica plants. 12 weeks for the C. fruticosus and C. physocarpus plants

Results of the Breeding System Experiments

There were no important differences in proportion of flowers that set fruit or the number of seeds produced per fruit

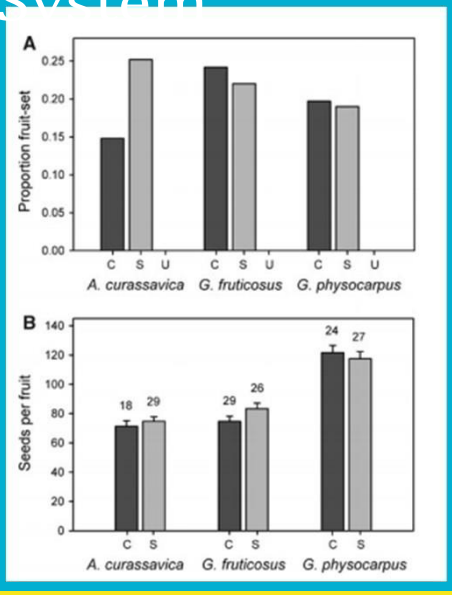


Fig. 1 Proportion fruit-set (a) and number of seeds per fruit (mean + SE, b) in the breeding system experiments for the three study species. Treatments are cross- pollinated (C), self-pollinated (S)

Results of the Hybridization Experiments

The two Gomphocarpus species and the A. curassavica did not set fruit

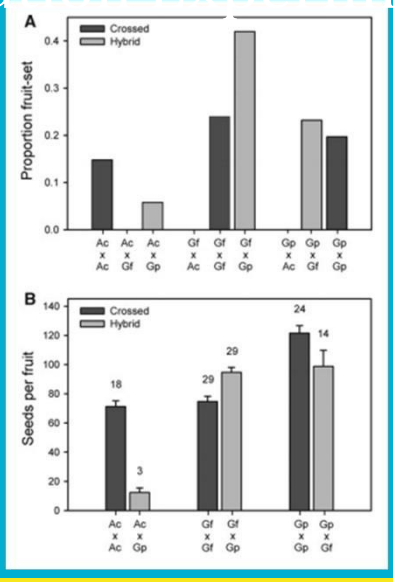


Fig. 2 Proportion fruit-set (a) and number of seeds per fruit (mean + SE, b) in the hybridisation experiments for the three study species.

Treatments are reciprocal hand-pollinations between A. curassavica (Ac), G. fruticosus (Gf) and

Results of the Performance of Intraspecific Progeny

A. curassavica had no difference in the time it took to germinate for self and cross pollination

G. fruticosus had high germination for self, crossed, and hybrid pollinated

G. physocarpus fewer self pollinated seeds germinated per fruitthan cross pollinated seeds

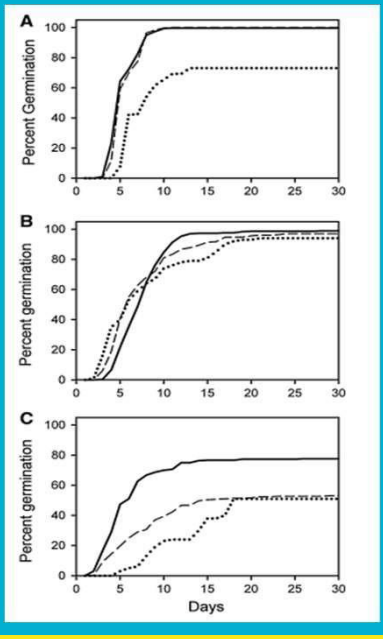


Fig. 3 Germination of seeds in progeny fitness trials for A. curassavica (a), G. fruticosus (b) and G. physocarpus (c). Treatments are cross-pollinated (solid line), self-pollinated (dashed line) and hybrid-

Results of the Performance of Hybrid Progeny

G. fruticosus and G. physocarpus self-pollinated seed survival was significantly lower than A. curassavica self-pollinated seeds

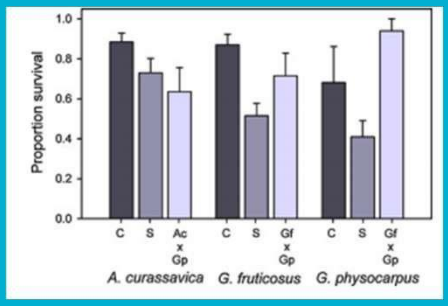
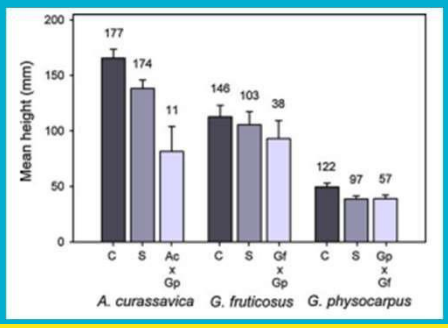


Fig. 4 Proportion of seedlings that survived (mean per fruit + SE) in progeny fitness trials for the three study species. Treatments are cross- pollinated (C), self-pollinated (S) and hybrid-pollinated where Ac is A. curassavica, Gf is G. fruticosus and Gp is G. physocarpus

G. physocarpus X G. fruticosus hybrid seeds grew to similar heights as intraspecific G. fruticosus crossed seeds and self pollinated seeds (lowest height)



Discussio

Results agree with Baker's

Other studies have similar results

Self-fertility is a significant contributor to the process of biological invasion

Role of selfing in invasion process is still a mystery

Accurately predicting species invasiveness is a long-term goal of invasion ecology

A.curassavica

G.fruticosus

G.physocarpu

Questions?

Works Cited

Ward, Megan, et al. “Modes of reproduction in three invasive milkweeds are consistent with Baker’s Rule.” Biological Invasions, vol. 14, no. 6, 2012, pp. 1237–1250., doi:10.1007/s10530-011-0152-2.

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https://commons.wikimedia.org/wiki/File:Asclepias_curassavica.jpg
https://commons.wikimedia.org/wiki/File:Gewone_braam (Rubus_fruticosus.jpg https://ecommons.cornell.edu/bitstream/handle/1813/1403/96-

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Q3rPZAhUK52MKHZj7AmUQ_AUICigB&biw=1920&bih=918#imgrc=O0qxF5C7FSlOxM:

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D.18. Student Sample 2: Formal writing assignment 1

- The Effects of Invasive *Asclepias curassavica* on the success of native *Asclepias incarnata* and *Asclepias* tuberosa
- The Success of Native Milkweed, *Asclepias incarnata* and *Asclepias tuberosa* when Grown Alone and with Invasive Milkweed, *Asclepias curassavica*

Introduction

Invasive species are defined as an exotic species whose introduction into a new area causes economic harm, environmental harm, or harm to human health. Invasive species are introduced into a new area where they cause detrimental impacts on the native species of the land. These invasive species tend to have better success rates including growth rate and life span when compared to native species. Invasion ecology attempts to look at the underlying reasons as to why these exotic species take the steps forward in the invasion process to become invasive species. Invasive species are described by characteristics such as their rapid growth and reproduction, high dispersal ability (assisted by humans), rapid adaptation to a similar environment, ability to acquire resources, and their freedom from predation pressure. The impact of invasive species on native species has been tested in many different scenarios. When examining invasive milkweed species known as *Asclepias curassavica* (tropical milkweed), research and experimental testing has found that *A. curassavica* has adapted to a similar environment, has undergone rapid growth, has reproduced strongly, and has actively cultivated. However, it is still unknown whether *A. curassavica* is a superior competitor in comparison to native species such as *Asclepias incarnata* (swamp milkweed) and *Asclepias tuberosa* (orange-butterfly milkweed). Here, the ability of *A. curassavica* to acquire resources more efficiently and therefore grow more successfully is at question. The present study describes in detail the success rate of each of the three species when grown in specific conditions. The observed data is then examined to determine which species is a superior competitor in terms of growth rate and survival in the same environmental conditions. *A. curassavica* will out compete native milkweed species such as *A. incarnata* and *A. tuberosa* when grown under the same conditions because of the invasive species ability to outcompete its opponents for resources.

Invasive species heavily impact everyday lives. There are over 50,000 invasive species in the United States alone. With this many invasions occurring in one country, the cost to combat these invasions has risen to over 120 billion dollars. This experiment takes a deeper look into the invasive milkweed species, *A. curassavica,* in Louisiana. *A. curassavica* is sold commercially in Louisiana due to its ability to grow year-round and its low maintenance for customers. Due to this invasive species' ability to grow year-round, this supplies a year-round food source for monarch butterflies, *Danaus plexippus* L., (Satterfield et al. 2015). This year-round supply of food reduces the butterfly's need to migrate; therefore, it increases disease prevalence among this species. This could potentially lead to an ecological trap for these monarchs. Winter-breeding becomes more frequent as it has in the southern United States in recent decades which has led to higher infection rates of non-migratory monarchs compared to migratory monarchs (Satterfield et at. 2015). *D. plexippus* L. larvae and adults are thought to benefit from the milkweed's chemical defenses

(Zalucki & Malcolm, 1999). These chemical defenses vary by species of milkweed. These inducible defenses include cardenolides, a group of toxic steroids with low molecular weights, which occur in the latex of almost all *Asclepias* species (Zalucki & Malcolm, 1999).

A. curassavica is displacing native species such as *A. incarnata* and *A. tuberosa*. Therefore, in this experiment, these three species will be examined. *A. incarnata* (swamp milkweed) is a common or native species in Louisiana. This species is found throughout a monarch butterflies migratory range. Swamp milkweeds are fragile, difficult to grow, and low in toxicity and latex. A milkweed's toxicity deals with the toxic steroids that disrupt sodium ions and potassium ions in the ATPase system in membranes. Latex is a sticky, viscous substance that is exuded upon tissue damage which can trap young monarchs and gums parts of their mouths. Both two defenses are considered inducible defenses which is a response activated through a previous encounter with a consumer or competitor that discusses some degree of resistance to subsequent attacks. These secondary defenses are effective against most generalist herbivores, yet it has since been verified by evidence that these toxins have little negative effect on specialist (Zalucki & Malcolm, 1999). *A. tuberosa* (orange-butterfly milkweed) is another common or native species in Louisiana and across parts of eastern North America (Wyatt, 2017). As well as swamp milkweed, orange-butterfly milkweed is also found throughout the monarch butterflies migratory range. These plants flower from May to July, but occasionally individual plants of this species are found to flower as late as September (Wyatt, 2017). This species has very hairy leaves, are moderately toxic, and produce little latex.

Methods

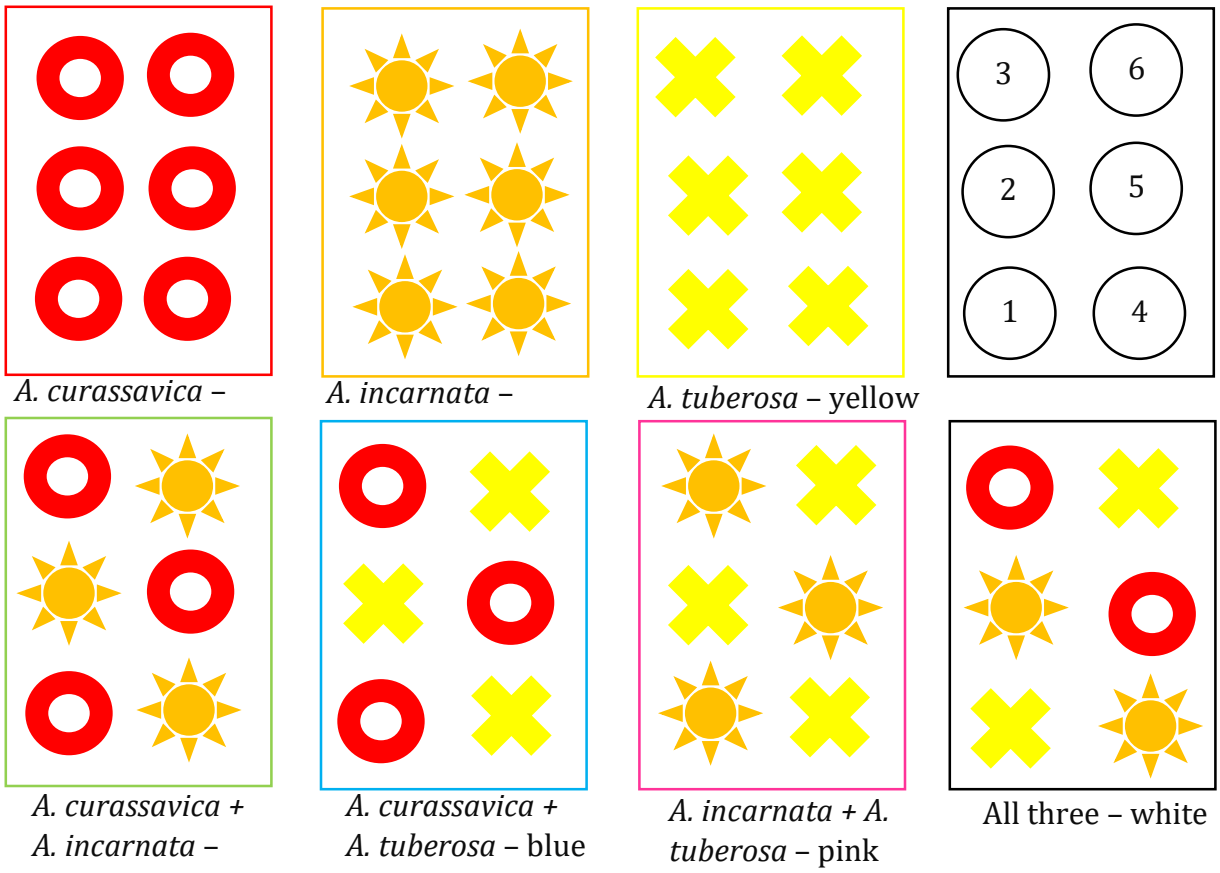
Species of *A. curassavica* were obtained from Cleggs Nursery, and species of *A. incarnata* and *A. tuberosa* were ordered from North Creek Nursery. The experiment was carried out in an enclosed LSU AgCenter Horticulture Greenhouse at Louisiana State University, Baton Rouge, Louisiana.

Once the plants were obtained and out of dormancy, we put a labeling system into place as shown in Table 1. Seven treatments were tested with seven replicates of each treatment. We used bussing tubs as a means of growing the plant. We then organized the plants in order in each tub as three by two as shown in Figure 1. The seven treatments included *A. curassavica* alone, *A. incarnata* alone, *A. tuberosa* alone, *A. curassavica* with *A. incarnata*, *A. curassavica* with *A. tuberosa*, *A. incarnata* with *A. tuberosa*, and all three grown together. The controls of the experiment were each of the species grown alone so that we could measure normal growth rates without competition in that given environment for each plant. To account for variation based on location of the plants in the green house, we paired plant treatments and the replicates were randomized.

Table 1. Labeling Data for Plant Species and Particular Tubs Planted in Bussing Tubs in Greenhouse Using 7 Colors

Legend	Species Type
Red	A. curassavica
Orange	A. incarnata
Yellow	A. tuberosa
Green	A. curassavica + A. incarnata
Blue	A. curassavica + A. tuberosa
Pink	A. incarnata + A. tuberosa
White	All Three

Figure 1. Planting Protocol to Insure Randomization with Replication when Planting Species in Greenhouse Bussing Tubs



Once the bussing tubs were randomly assigned, we planted all the milkweed species in accordance to the assignment. We then placed the labelled sticks in front of each plant to specify each plant to get accurate and precise measurements at each data collection. Each species was planted in Scotts Osmocote 14-14-14 fertilizer with around 40 pounds of soil per bussing tub. The green house is equipped with a sprinkler system that waters the plants daily at 7 am for 30 minutes using a drip system. We planted the species on January 30, 2018. The initial data collection was also taken on January 30, 2018 which is where we collected our initial data.

During the initial data collection, we collected the following pieces of data: number of stems, number of leaves, plant height, lengths of each stem, and whether the species survived. We collected the data as a group using a ruler. The measurements were taken in centimeters. We used the ruler to measure the stem(s) height per plant. After each height was recorded, we counted the number of leaves on each plant. We then determined whether each plant had survived or not initially. Data collection 2 took place on March 13, 2018. This was the second and final data collection. In this collection, the same questions were asked, and the same measurements were taken as in data collection one; however, in the final collection, we also collected tube weight before, tube weight after, number of trichomes, above ground bio mass, below ground biomass, and number of flowers. These categories of data could not be collected initially as the plants had not had enough time to grow and develop to determine these factors.

The data analysis used for this experiment was JMP by SAS. This data was collected and used to determine the invasive species', *A. curassavica*, ability to compete with the two-native

species, *A. incarnata* and *A. tuberosa*. Through the success rates of each species grown individually when compared to each species grown together, we can determine which species is a superior competitor when competing for the same resources (soil, water, growth space, etc.).

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D.19. Student sample 3: Formal writing assignment 2

The negative effects of invasive tropical milkweed (*A. curassavica*) on native plant species (*A. incarnata* and *A. tuberosa*) and monarch butterfly populations.

Results

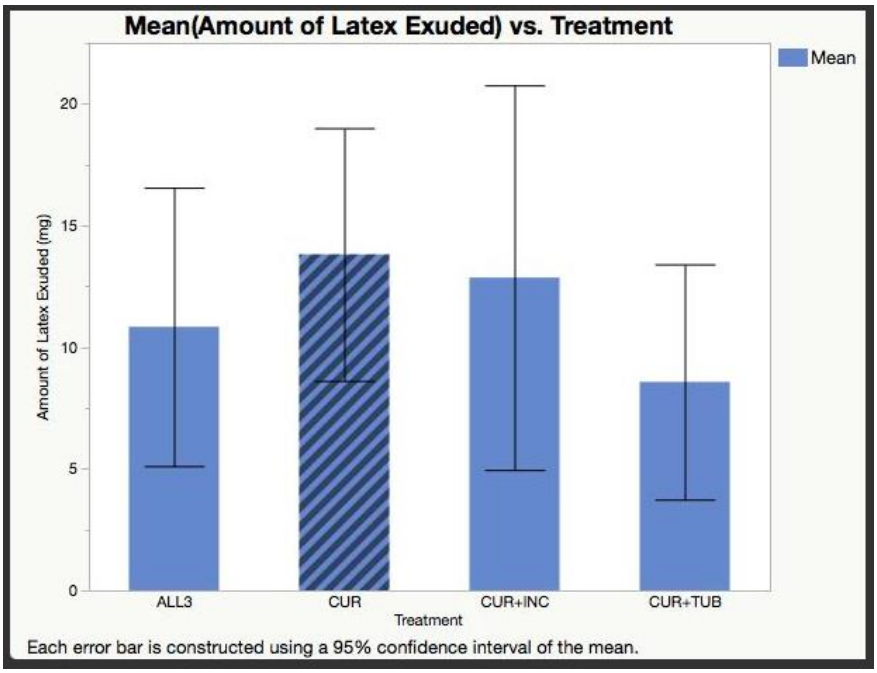

Figure 1: The bar graph above shows the ANOVA test for the average amount of latex exuded by each treatment of plants. *A. curassavica* grown alone, *A. curassavica* grown with *A. incarnata*, *A. curassavica* grown with *A. tuberosa*, and all three grown together. ($F_{3,92}$= 0.6195, p=0.604, R2=0.0198, Figure 1). The *A. curassavica* grown alone resulted in the most latex exuded. The error bars, that use a 95% confidence interval of the mean, overlap extensively.

The Effect of Plant Height on the amount of Latex Exuded

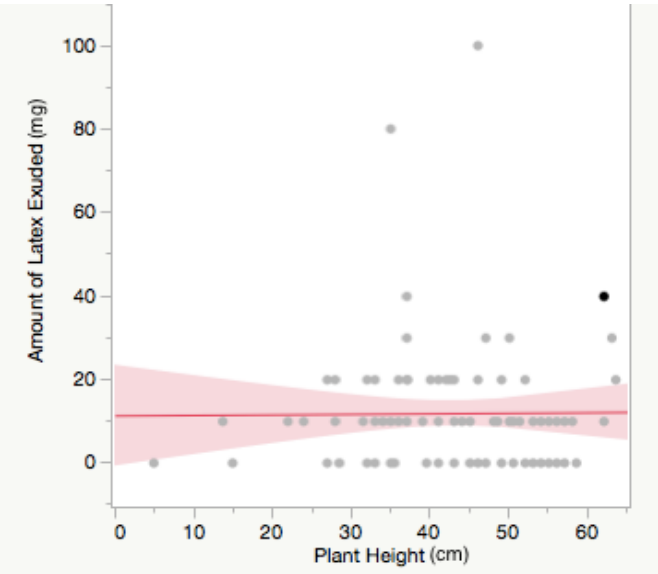

Figure 2: The effect plant height (cm) has on the amount of latex exuded (mg). The slope of the graph is horizontal showing no clear correlation of plant height and amount of latex exuded. Plant height is not significantly correlated to the amount of latex exuded ($F_{3,92}$=0.0086, p=0.9264, R2=9.13e-5, Figure 2)

For the independent variable "treatment," the results of the ANOVA detected no significant difference with the dependent variable "amount of latex exuded." The 95% confidence interval of the mean, shown in Figure 1, shows that there were no significant differences between any of the treatments, which are further supported by a p-value greater than the alpha 0.05 (p-value=0.604). The F-statistic was under one at $F_{3,92}$= 0.6195, and our R squared statistic was relatively low (R2=0.0198).

When performing a regression tests for the variables "plant height" (independent) and "amount of latex exuded" (dependent), the lack of slope in our results, shown in Figure 2, showed no relationship or correlation. The F-statistic was greatly under one ($F_{3,92}$=0.0086), and the R squared value was incredibly small (R2=9.13e-5).

Discussion

The results rejected the first hypothesis, that the amount of latex exuded affects the plants performance when planted with other native species. The performance of *A. curassavica* in terms of latex exudation was insignificantly changed when forced to compete with native species *A. incarnata* and *A. tuberosa*. This shows one reason why *Asclepias curassavica* is a successful invader because it's defense mechanisms are barely affected by the presence of other plant species. The unaltered amount of latex exuded when *Asclepias curassavica* is planted with other

species means that *Asclepias curassavica's* ability to deter predators is unaffected by the presence of native species making *Asclepias curassavica* more tolerant to herbivory. This makes *Asclepias curassavica* a better competitor than the native species and is crucial component of what allows *Asclepias curassavica* to become a successful invasive species. Another crucial component of invasive species we observed was plant height.

The second hypothesis was also rejected by our results for this experiment, that plant height affects the amount of latex exuded. *A. curassavica* latex exudation was not significantly affected by plant height, regardless of the treatment heights there was an insignificant difference when comparing the amount of latex exuded by each. The insignificant difference between plant height and latex exudation provides evidence to support that there is no correlation between the two variables. This suggests that plant height isn't the best predictor of defense production although it is ultimately beneficial in resource acquisition (Berntson et al. 2000). Increased plant height, as seen in *Asclepias curassavica,* is still a characteristic that affects a species ability to become invasive as seen in a meta-analysis comparing invasive and native species (Kleunen et al.2010).Since there is no correlation between plant height and latex exudation, regardless if they are native or invasive, all of the plants are equally susceptible to herbivory in terms of protection that the latex provides.

In this experiment, *Asclepias curassavica* outcompeted the native *Asclepias tuberosa* and *Asclepias incarnata* providing a vital example of what characteristics makes a species a successful invader. Increased defenses towards herbivory, as seen in *Asclepias curassavica*, is a prime example of how invasive plants are able to protect themselves from predators even in the presence of other competitors. Invasive species that are able to defend themselves from predators, can continue to grow and compete for resources in a new area. In an environment with scarce resources, invasive species limit the amount of resources native species are able to intake and negatively affect the native species populations and ultimately affect the entire ecosystem (Vila et al. 2011). Specifically, in this experiment, *Asclepias curassavica* impeded the growth of native milkweed populations that could lead to negative effects on Monarch butterfly populations. More research will be needed to determine exactly what effects *Asclepias curassavica* has on Monarch Butterflies.

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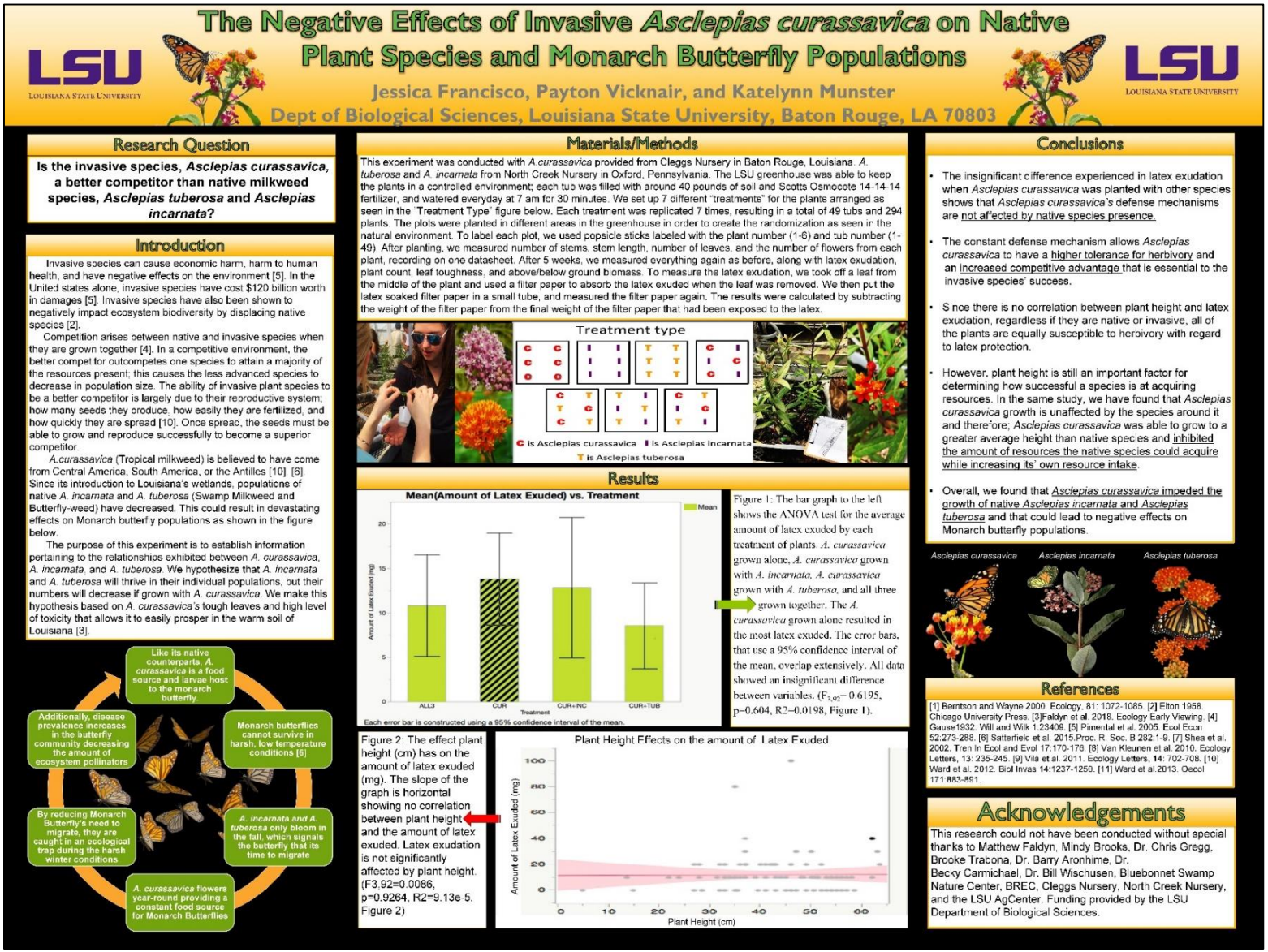
D.20. Student sample 4: 1st Place overall student poster



D.21. Student sample 5: 3rd Place overall student poster

How the Presence or Absence of Native and Invasive Species affects Native Milkweed Fitness

Mary J. Melancon, Ruby M. Harriford, and Renee O. Viator
Dept. of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

Introduction

- Invasive species start as an exotic species, but if the exotic species cause ecological, economic, and/or human harm, then the exotic species is considered invasive (3).
- *Asclepias curassavica* is displacing native species such as *Asclepias incarnata* and *Asclepias tuberosa* (4).
- *A. incarnata* (swamp milkweed) is a native species in Louisiana which is found throughout a monarch butterflies migratory range. Swamp milkweeds are fragile, difficult to grow, and low in toxicity and latex (5).
- *A. tuberosa* (orange-butterfly milkweed) is another native species in Louisiana which is also found throughout the monarch butterflies migratory range. This species has very hairy leaves, is moderately toxic, and produces little latex (5).
- The tropical milkweed also produces food for the monarch butterflies (*Danaus plexippus*) year round, thus preventing the butterflies from migrating in the winter. This causes the monarch population to decrease as more monarchs are dying from the harsh winter conditions rather migrating south (4).

Research Question

How is *A. incarnata*'s belowground biomass affected by its growing conditions with or without native and invasive species?

Hypotheses

1) Invasive plant species affects the belowground biomass of native plant species.
2) The belowground biomass of a plant will affect the plant's height.

Acknowledgements

We would like to thank Matthew Faldyn, Mindy Brooks, Dr. Chris Gregg, Brooke Trabona, Dr. Barry Aronhime, Dr. Becky Carmichael, Dr. Bill Wischusen, Bluebonnet Swamp Nature Center, BREC, Cleggs Nursery, North Creek Nursery, and the LSU AgCenter. Funding provided by the LSU Department of Biological Sciences.

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Results

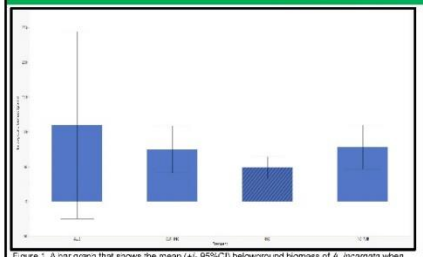


Figure 1. A bar graph that shows the mean (+/- 95%CI) belowground biomass of *A. incarnata* when it is grown with other native and invasive species.

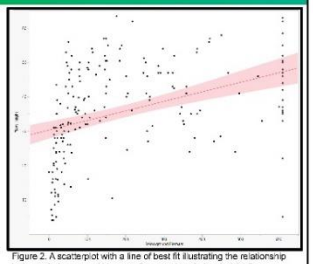


Figure 2. A scatterplot with a line of best fit illustrating the relationship between belowground biomass (in grams) and plant height (in centimeters).

For ANOVA:

•Belowground biomass does not differ among the treatments ($F_{3,81}$=1.78, p=0.157, Figure 1).

•None of the treatments have a significantly different belowground biomass than the others (p values range from 0.160 to 0.999).

For Regression:

•Belowground biomass is significantly correlated to the plant height ($F_{1,196}$=37.2622, p<0.0001, R^2=0.160, Figure 2).

•As belowground biomass increases by 1g, the plant height increases by 0.0278458cm. The R^2 value for this regression was equal to 0.160, which is interpreted as 16% of the Y variable is determined by the X variable.

A. curassavica before the final data collection. March 13, 2018.

Multiple steps in the final data collection process.

Conclusions

For ANOVA Testing:

- The belowground biomass of *A. incarnata* is not affected when grown with the invasive species, *A. curassavica*, and/or other native species, *A. tuberosa*, meaning that invasive milkweed is not as much of a hindrance on the native milkweed belowground biomass as initially predicted.
- Other studies found that competitive effects of belowground biomass appeared to be more severe at high levels of resource availability (1).
- Abiotic factors, that were not collected through data collections, could be what affects the growth of *A. incarnata*'s belowground biomass, or invasive species, *A. curassavica*, is not as superior of a competitor as some studies may show.

For Regression Testing:

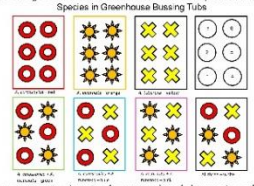
- As a plant's roots grow bigger and spread throughout the soil, that plant can obtain more nutrients from the soil allowing it to grow taller.
- Other studies have shown that when certain factors stimulate biomass growth, plant height growth is also stimulated resulting in a positive correlation between the two (2).
- For invasive species (*A. curassavica*), if their below ground biomass increases at faster rates than native species (*A. incarnata* and *A. tuberosa*), it could out-grow native species in height, and therefore, the invasive species could out compete the native species in their own ecosystem.

Future Improvements:

- Allowing more time for growth would allow all species of milkweed to come out of dormancy; therefore, the results would be able to be concluded using more species.
- Multiple data collections would allow for continuous patterns of growth of each species for more accurate determinations.

Methods

Figure 3. Planting Protocol to Insure Randomization with Replication when Planting Species in Greenhouse Bussing Tubs



- There were seven treatments: *A. curassavica*, *A. incarnata*, and *A. tuberosa* were planted themselves. *A. curassavica* and *A. incarnata* were planted together, *A. curassavica* and *A. tuberosa* together, and *A. incarnata* and *A. tuberosa* together. All three were planted together. There were seven replicates.
- The controls of the experiment were each of the species grown alone so that we could measure normal growth rates without competition in that given environment for each plant.
- To account for variation based on location of the plants in the green house, we paired plant treatments and the replicates were randomized.
- During the initial data collection, we collected the following pieces of data: number of stems, number of leaves, plant height, lengths of each stem, and whether the species survived. We collected the data as a group using a ruler. The measurements were taken in centimeters.
- In the final collection, along with the same initial types of data collected, we also collected tube weight before, tube weight after, number of trichomes, aboveground biomass, belowground biomass, and number of flowers.
- We used JMP to analyze the data we collected.

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IRB Application

1 message

Institutional R Board <irb@lsu.edu> Tue, Jan 23, 2018 at 9:35 AM
To: Matthew J Faldyn <mfaldy1@lsu.edu>
Cc: William Wischusen <ewischu@lsu.edu>

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Elizabeth

LSU

Elizabeth Cadarette
IRB Coordinator
Office of Research and Economic Development
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office 225-578-8692 | fax 225-578-5983
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Elizabeth Cadarette
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Louisiana State University
130 David Boyd Hall, Baton Rouge, LA 70803

VITA

Matthew Faldyn, from Katy, Texas, has been interested in understanding our natural world for as long as he can remember. His interest in wildlife and ecology was fostered by his family, specifically his father, who ensured that he was raised to ask and appreciate our natural world through fishing, hunting, hiking, naming cattle and plant species, and simply just enjoying nature. His passion for ecological research, with a specific concern for climate change, was formalized while completing his undergraduate degree at Louisiana State University (LSU), Baton Rouge, Louisiana, through various courses and projects. After completing his undergraduate degree, he began pursuing his Master's degree. In the Spring of 2016, Matthew transferred his Master's degree progress into the Ph.D. program at LSU. For his dissertation, he quantified how climate change will impact species and their interactions using the charismatic monarch butterfly (*Danaus plexippus*) – milkweed (*Asclepias* sp.) while also improving research experiences for undergraduate students. In August 2019, Mathew will be moving to Southern California to serve as a Visiting Assistant Professor at the W. M. Keck Science Department for Claremont-McKenna College, Pitzer College, and Scripps College. Here, he will be teaching a variety of courses while also participating in various research opportunities. Matthew ultimately hopes that his past and future work will serve as both a valuable contribution to the understanding of our natural world and also help students engage with science through unique and memorable avenues.